'TITLE OF THE INVENTION
SPLICE VARIANTS OF HUMAN VOLTAGE-GATED CALCIUM CHANNEL $\alpha_2\delta$-2 SUBUNIT DESIGNATED - $\alpha_2\delta$2-a AND $\alpha_2\delta$2-b.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/463,826, filed April 18, 2003, the contents of which are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

The present invention relates to novel nucleic acid molecules, encoded proteins, vectors, host cells transformed therewith, antibodies reactive with said proteins, as well as pharmaceutical compositions. Methods of using any of the foregoing, e.g., methods for screening for candidate agonists or antagonists utilizing the novel protein isoforms are also contemplated by the present invention.

Calcium channels were discovered in 1958 by Fatt and Ginsborg when they explored the ionic basis of a Na+ -independent action potential in crab muscle. The most unique and crucial role of Ca^{2+} channels is to translate the electrical signal on the surface membrane into a chemical signal within the cytoplasm, which, in general, increases the intracellular second messenger Ca^{2+}, which, in turn, activates many crucial intracellular processes including contraction, secretion, neurotransmission and regulation of enzymatic activities and gene expression. Tsien et al., (1988), Trends Neurosci., vol. 11, pp. 431-438. As might be expected from their central role in signal transduction, Ca^{2+} channels are tightly regulated by a range of signal transduction pathways in addition to regulation by their intrinsic, voltage-dependent gating processes.

Continuing studies have revealed that there are multiple types of Ca^{2+} currents as defined by physiological and pharmacological criteria. See, e.g., Catterall, W.A., (2000) Annu. Rev. Cell Dev. Biol., 16:521-55; Llinas et al, (1992) Trends Neurosci, 15;351-55; Hess, P. (1990) Ann. Rev. Neurosci. 56:337; Bean, B. P. (1989) Ann. Rev. Physiol. 51:367-384; and Tsien et al. (1988) Trends Neurosci. 11:431-38. In addition to exhibiting distinct kinetic properties, different Ca^{2+} channel types can be localized on different regions of a cell with complex morphology. Finally, Ca^{2+} channels in different tissues display different pharmacological profiles, suggesting the possibility of drugs selective for particular organs.

The most common type of calcium channel is the voltage-gated calcium channel also known as voltage dependent calcium channels (VDCCs). Electrophysiological studies on

invertebrate preparations suggest the presence of multiple types of voltage-dependent calcium channels, reviewed by Hille, B., (1992), In: Ion Channels of Excitable Membranes, 2nd Ed., Sinauer, Sunderland, Mass.). These can be broadly grouped into high-voltage (HVA) and low-voltage activated (LVA) channels (Marais et al., (2001) Molecular Pharmacology, 59: 1243-1248). The calcium currents produced by the high-threshold channels can be further sub-grouped into L-, R-, P/Q-, and N-type, depending on their biophysical characteristics. All are expressed in brain. See, e.g., Dunlap et al. (1995) Trends Neurosci. 18, 89-98; Varadi et al. (1995) Trends Pharmacol. Sci. 16, 43-49; Nooney et al. (1997) Trends Pharmacol. Sci. 18, 363-371; Perez-Reyes et al. (1998) Nature 391, 896-900. Voltage-gated calcium channels are generally characterized as being present in all "excitable" cells in animals, exemplified by neurons of the central nervous system, peripheral nerve cells, and muscle cells, including those of skeletal muscles, cardiac muscles, and venous and arterial smooth muscles.

The high-voltage-activated Ca^{2+} channels have been characterized biochemically. Structurally, most high-voltage-activated Ca^{2+} are complexes of a pore-forming $\alpha 1$ subunit of a ~190-250 kDa; a transmembrane, disulfide-linked complex of $\alpha 2$ and δ subunits; an intracellular β subunit. See, e.g., De Waard et al. Structural and functional diversity of voltage-activated calcium channels. In Ion Channels, (ed. T. Narahashi) 41-87, (Plenum Press, New York, 1996); Catterall, W. A., (1991a), Cell, vol. 64, pp. 871-874; and Catterall, W. A., (1991b), Science, vol. 253, pp. 1499-1500. Until recently, only a single γ subunit in skeletal muscle had been described by Eberst et al., (1997) Pflugers Arch. 433: 633-7. To date, 8 γ subunits have been identified.

The α_1 subunit contains the binding sites for selective channel antagonists and is composed of four homologous repeats (I–IV) each comprising six transmembrane segments (S1–S6). Seven genes have been identified for the α_1 subunits of HVA channels and three for LVA channels, reviewed in Hofmann et al., (1999), Rev. Physiol. Biochem. Phamacol. 139:33-87; Lacinova et al.,(2000) Gen. Physiol. Biophys., 19: 121-36). The surface of voltage-gated calcium channels comprises molecular pores that "open" in response to the depolarization of the membrane voltage, which, in turn, allows for the selective influx of Ca2+ ions from an extracellular environment into the interior of a cell. The "opening" of the pores essentially requires a depolarization to a certain level of the potential difference between the inside of the cell bearing the channel and the extracellular medium bathing the cell. The rate of influx of Ca.sup.2+ into the cell depends on this potential difference. When the accumulating Ca^{2+} reaches a sufficient concentration, it can activate ion channels such as Ca^{2+} activated K+ channels that allow positive charge out the cell and thereby repolarize the membrane. It can be

seen how calcium channels serve as elements that can sense, amplify, and terminate electrical signals.

The $\alpha 2\delta$ family consists of four genes. The $\alpha 2\delta$-4 subunit was recently described by Qin, N. et al., Mol. Pharmacol. 62(3) 485-496 (2002). The $\alpha 2\delta$ subunit ($\alpha 2\delta$-1) was first identified in biochemical studies of skeletal muscle L-type Ca^{2+} channels. Perez-Reyes, E., and Schneider, T, (1995), Kidney Int. 48: 1111-1124; Ellis et al., (1988), Science, 241:1661-1664. Research data further suggests that it is also a part of the cardiac L-type and neuronal N-type channels (Schmid et al., (1986), Biochemistry, 25: 3492-3495). To date, five tissue-specific splice variants of the $\alpha 2\delta$ subunit have been identified (Angelotti and Hofmann, (1996) Febs Lett., 397: 331-337). The different splice variants are hypothesized to arise from various combinations of three alternatively spliced regions that result in five isoforms that are expressed in a tissue-specific manner (Angelotti and Hofmann, supra). Two new $\alpha 2\delta$ family members $\alpha 2\delta$-2 and $\alpha 2\delta$-3 have been identified in human and mouse (Klugbauer et al.,(1999), J. Neuroscience, 19: 664-691). These isoforms have been found to be 56 and 30% homologous to $\alpha 2\delta$-1 at the amino acid level and share a number of structural motifs. The subunits have similar hydrophobicity profiles and all contain several potential N-glycosylation sites. Northern analysis has shown that $\alpha 2\delta$-1 is ubiquitously expressed, $\alpha 2\delta$-2 is found in several tissues including brain and heart, and $\alpha 2\delta$-3 is brain-specific (Klugbauer et al., supra).

Structurally, the $\alpha 2\delta$ subunit is a heavily glycosylated 175 kDa protein that is post translationally cleaved to yield a disulfide-linked $\alpha 2$ and δ protein (DeJongh et al., (1990) J. Biol. Chem., 265:14738-41; Jay et al., (1991) J. Biol. Chem., 266:3287-93). Structural studies have shown that the δ part anchors the $\alpha 2$ protein to the membrane via a single transmembrane segment (Gao et al., (2000), J. Biol. Chem., 16:12237-12242; Gurnett et al., (1996) Neuron, 16: 431-40). The data further suggest that the δ domain, which contains the only transmembrane segment of $\alpha 2\delta$ complex, harbors the regions important for the shift in voltage-dependent activation, steady-state inactivation, and the modulation of the inactivation kinetics (Marais et al. supra; Felix et al., (1997) J. Neurosci., 17: 6884-6891). According to Gee et al., (1996), J. Biol. Chem., 271: 5768-5776, gabapentin (GBP), an antiepileptic drug, has been shown to bind to $\alpha 2\delta$-1 of voltage activated calcium channels. Subsequently, the $\alpha 2\delta 2$ subunit was also shown to bind gabapentin with high affinity (Marias et al., Mol. Pharm. 59: 1243-1248 (2001)). Because voltage activated channels are involved in controlling the electrical excitability of neurons, it has been postulated that this drug reduces calcium current by modulating $\alpha 1$ indirectly through its association with $\alpha 2\delta$-1 or $\alpha 2\delta 2$ (Gee et al., supra; Marias et al. supra).

The VDCC's are implicated in a variety of biological functions, such as presynaptic neurotransmitter release and protein signaling within the cell. See, e.g., Bito et al.

(1997) Curr. Opin. Neurobiol. 7, 419-429; Dunlap et al., supra. Because calcium channels are present in various tissues and have a central role in regulating intracellular calcium ion concentrations, these channels, as noted below, have been implicated in various human diseases including, but not limited to disorders of the central nervous system, cardiovascular disease etc.

For example, cellular calcium homeostasis plays an essential part in the physiology of nerve cells. The intracellular calcium concentration is about 0.1 uM compared with 1 mM outside the nerve cell. This steep concentration gradient (X10,000) is regulated primarily by voltage-gated calcium channels. Several pathologies of the central nervous system involve damage to or inappropriate function of voltage-gated calcium channels. In cerebral ischaemia (stroke) the channels of neurons are kept in the open state by prolonged membrane depolarizations, producing a massive influx of calcium ions. This, in turn activates various calcium/calmodulin dependent cellular enzyme systems, e.g. kinases, proteases and phospholipases. Such prolonged activation leads to irreversible damage to nerve cells. Changes to calcium influx into neuronal cells may also be implicated in conditions such as epilepsy, stroke, brain trauma, Alzheimer's disease, multiinfarct dementia, other classes of dementia, Korsakoff's disease, neuropathy caused by a viral infection of the brain or spinal cord (e.g., human immunodeficiency viruses, etc.), amyotrophic lateral sclerosis, convulsions, seizures, Huntington's disease, amnesia, or damage to the nervous system resulting from reduced oxygen supply, poison or other toxic substances (See e.g., Goldin et al., U.S. Pat. No. 5,312,928).

Likewise, changes to calcium influx into cardiovascular cells may be implicated in conditions such as cardiac arrhythmia, angina pectoris, hypoxic damage to the cardiovascular system, ischemic damage to the cardiovascular system, myocardial infarction, and congestive heart failure (Goldin et al., supra). Other pathological disease states associated with dysfunctional calcium channels, e.g., elevated intracellular free calcium levels include muscular dystrophy and hypertension (Steinhardt et al., U.S. Pat. No. 5,559,004).

A clinical connection between voltage-dependent calcium channels and lung cancer is evidenced by the Lambert-Eaton myasthenic syndrome, characteristic in some small cell lung cancer patients. Lambert-Eaton myasthenic syndrome is a human antoimmune disorder that impairs neuromuscular transmission resulting in the aberrant release of Acetycholine from motor nerve terminals due to a defective calcium channel. Reportedly, patients afflicted with this disease develop antibodies (presumably initiated by expression of the channel proteins in their small cell lung cancer) that react with voltage-gated calcium channel polypeptides thereby blocking depolarization-induced Ca^{2+} influx, leading to the myasthenia. The availability of purified recombinant calcium channel subunit isoforms detailed herein makes possible

immunoassays for the diagnosis of such diseases and use of the functional proteins in designing effective therapeutics specific for this disease.

Likewise, the work by Toyota et al., (1999) Cancer Res., 59: 4535-4541, concludes that the $\alpha 2\delta$-2 subunit functionally interacts with the T-type channel subunit $\alpha 1G$. The data implicates this interaction in various human tumors such as colorectal cancers, gastric cancers, and acute myelogenous leukemias. Of significant importance is the suggestion by Gao et al., (2000), J. Biol. Chem., 16:12237-12242 linking the $\alpha 2\delta$-2 gene with lung, breast and other cancerous diseases, which further corroborates the finds by Toyota et al.

Similarly, improper functioning of calcium channels has also been implicated in improper pain transmission in the central nervous system, and epilepsy. Epilepsies are a heterogeneous group of disorders characterized by recurrent spontaneous seizures affecting 1% of the population. In recent years several human genes encoding ion channels have been implicated in benign familial neonatal convulsions (Charlier et al. (1998) Nature Genet. 18, 53-55; Singh et al. (1998) Nature Genet. 18, 25-29; Biervert et al. (1998) Science 279, 403-406). Significantly, in at least two mouse models of epilepsy – tottering and lethargic – the defect has been connected to a gene encoding a neuronal calcium channel subunit (Marais et al., supra; Burgess et al. (1997) Cell 88, 385-392; Fletcher et al. (1996) Cell 87, 607-617). Likewise, the mouse homolog has been reported to be a candidate for the *ducky* epileptic phenotype. Gao et al., supra. In addition, Gee et al., supra, have implicated a $\alpha 2\delta$-1 isoform as the *in vivo* target for the antiepileptic drug gabapentin, which is thought to inhibit calcium currents in brain neurons. See also, Klugbauer et al., supra; Wang et al., (1999) Biochem. J., 342;313-320; and Stefani et al., (1998), Neuropharmacology, 37: 83-91. Importantly, as detailed below, the herein disclosed $\alpha 2\delta 2$ isoforms will find utility in the identification of therapeutics for treating voltage-gated calcium channel mediated pathologies considering that the isoforms of the invention share at least one property .e.g., binding affinity for gabapentin similar to prior art isoforms. Gee et al, supra.

As well, there is a need for a better understanding of the structure and function of calcium channels, which, in turn would permit identification of substances that, in some manner, modulate the activity of calcium channels and that have potential for use in treating such disorders. That mutations of several channel proteins have been shown to be a causative factor in neurological disorders, is well known, thereby making the calcium channel subunits target for therapeutic interventions. See, e.g., Marais, supra and Burgess and Noebels, (1999) Epilepsy Res., 36:111-122.

An understanding of the pharmacology of compounds that interact with calcium channels in other organ systems, such as the central nervous system ("CNS"), will greatly aid in

the rational design of compounds that specifically interact with the specific subtypes of human calcium channels to have desired therapeutic effects, such as in the treatment of neurodegenerative and cardiovascular disorders. Such an understanding together with the ability to rationally design therapeutically effective compounds have been hampered by an inability to independently determine the types of human calcium channels and the molecular nature of individual subtypes, particularly in the CNS, and by the unavailability of pure preparations of specific channel subtypes to use for evaluation of the specificity of calcium channel-effecting compounds. Thus, the identification of nucleic acid molecules encoding human calcium channel subunits coupled with the use of such molecules for expression of the encoded calcium channel subunits subsequent use in of the functional calcium channels would aid in screening and design of therapeutically effective compounds.

In addition to the implication of calcium channels in animal (including human) diseases, a number of compounds which are currently used for treating various dysfunctional calcium channel diseases in animals (including humans) are believed to exert their beneficial effects by modulating the functions of voltage-dependent calcium channels present in cells. Nonetheless, there is a paucity of understanding of the pharmacology of compounds which interact with calcium channels. This paucity of understanding, together with the limited knowledge in the art of the human calcium channel types, the molecular nature of the human calcium channel subtypes, and the limited availability of pure preparations of specific calcium channel subtypes to use for evaluating the efficacy of calcium channel-modulating compounds has hampered the rational testing and screening of compounds that interact with the specific subtypes of human calcium channels to have desired therapeutic effects.

Sequence and structural homology exists between the novel $\alpha 2\delta$-2 isoforms detailed herein and the reference isoform designated as the "reference protein." Significant, the development of new therapeutic strategies against, and the creation of new analytical tools for, a better understanding of diseases characterized by aberrant voltage regulated calcium influx are greatly desired. It is particularly desirable to provide such tools and therapies that are highly specific to a target gene and protein, which at the same time, because of their specificity, do not substantially affect other proteins or body functions.

Consequently, the discovery of the herein disclosed isoforms of the $\alpha 2\delta$-2 subunit will allow for the development of therapeutic compounds specific for pathologies mediated by the $\alpha 2\delta$ subunit of the voltage-gated calcium channel and/or characterized by aberrant voltage regulated calcium influx thereby satisfying a long-sought need for such therapies and tools.

SUMMARY OF THE INVENTION

The present invention is based on the finding of novel, naturally occurring splice variants of the human α2δ-2 subunit of voltage-gated calcium channel which plays a role in voltage-gated calcium influx. These are naturally occurring sequences obtained by alternative splicing of the known α2δ-2 genes. A sequence of the reference gene was published as GeneBank accession # AF042792 (reference sequence), as well as (Gao, et al., supra).

The novel splice variant encoding sequence(s) of the invention are not merely truncated forms or fragments or mutation (for example by insertion) of the known gene, but rather novel sequences, which naturally occur within the body of individuals and may thus have physiological relevance. The inventors have discovered that one of the herein disclosed nucleotide sequences encoding one of the α2δ-2 calcium channel subunit isoforms (splice variant #1) referred to herein as α2δ2-a contains a three nucleotide insert encoding a single glutamine (Q) residue after amino acid 863 relative to the reference sequence, while splice variant#2 (α2δ2-b) comprises a 81 nucleotide insert thereby resulting in an encoded protein comprising an insert of 27 amino acids between amino acids 1099 and 1100 of the reference sequence.

The novel α2δ-2 calcium channel subunit isoforms of the invention are meant to define distinct but closely related calcium channel proteins, i.e., especially considering the degree of sequence homology between the novel α2δ-2 isoforms detailed herein and the reference protein. Specifically, the α2δ2-a shares an amino acid sequence homology of approximately 99.9% and nucleotide sequence similarity of 99.9% with the prior art calcium channel protein α2δ-2 subunit isoform (reference sequence) as it relates to splice variant #1. The novel splice variant #2 shares a 97.6% amino acid sequence similarity with the reference sequence and a 97.7 % nucleotide sequence similarity with the reference sequence. Consequently, the novel isoforms are also thought to be functionally similar to the prior art α2δ-2 subunit isoforms based not only on the structural similarity between the isoforms of the invention and the reference protein but also on the binding affinity of the isoforms of the invention for gabapentin.

The prior art is replete with information detailing the therapeutic utilities in recombinant materials derived from the DNA of the numerous calcium channels including the various isoforms of the α2δ subunit. See, e.g., Gee et al.; Gao, et al.; Burgess and Noebels, and Klugbauer et al. which collectively detail the uses of the α2δ-1 subunit protein including its isoforms both as a therapeutic and a diagnostic tool.

Considering the high degree (99.9% amino acid sequence similarity – splice variant #1 and 97.7% amino acid sequence similarity-splice variant #2) of sequence homology in

the primary sequence between the reference α2δ-2 subunit isoform sequence and the novel isoform sequences disclosed herein, it is believed that compositions comprising the novel splice variants or a fragment or derivative thereof may be administered to a subject to treat or prevent a pathological disorder characterized by a dysfunctional voltage-gated calcium channel subunit mediated by a α2δ-2 calcium channel isoform subunit. As such, the novel isoforms of the invention may find use, *inter alia*, in treating a number of α2δ-2 subunit mediated pathologies including epilepsy, colorectal cancers, gastric cancers, acute myelogenous leukemias as well as lung and breast cancers. See Gao et al., *supra*.

The term "alternative splicing" in the context of the present invention and claims refers to: intron inclusion, exon exclusion, addition or deletion of terminal sequences in the splice variants as compared to the reference sequences.

In accordance with the above, there are provided isolated nucleic acid molecules, comprising a sequence of nucleotides that encodes a functional human voltage-gated calcium channel α2δ-2 subunit polypeptide or a molecule comprising a sequence having at least 90% identity to any one of SEQ ID NO:1 or 3. Preferably, the isolated nucleic acid molecules comprise the nucleotide sequence as set forth in SEQ ID NO:1 or 3, wherein SEQ ID NO:1 comprises a coding portion encoding α2δ2-a, while SEQ ID NO:3 encodes splice variant α2δ2-b.

The invention further provides nucleic acid molecule(s) comprising a nucleotide sequence which is complementary to that of SEQ ID NO:1 or SEQ ID NO:3, or complementary to a sequence having at least 90% identity to said sequence or a fragment of said sequence. The complementary sequence may be a DNA sequence which hybridizes with any one of SEQ ID NO:1 or 3 or hybridizes to a portion of that sequence having a length sufficient to inhibit the transcription of the complementary sequence. The complementary sequence may be a DNA sequence which can be transcribed into an mRNA being an antisense to the mRNA transcribed from any one of SEQ ID NO:1 or 3 or into an mRNA which is an antisense to a fragment of the mRNA transcribed from any one of SEQ ID NO:1 or 3 which has a length sufficient to hybridize with the mRNA transcribed from any one SEQ ID NO:1 or 3, so as to inhibit its translation. The complementary sequence may also be the mRNA or the fragment of the mRNA itself.

The present invention further provides nucleic acid molecule comprising a nucleotide sequence which encode the amino acid sequences of SEQ ID NO:2 or 4, including fragments and homologues of the amino acid sequences. Due to the degenerative nature of the genetic code, a plurality of alternative nucleic acid sequences beyond those depicted in any one of SEQ ID NO:1 or 3, can code for the amino acid sequences of the invention. Consequently, those alternative nucleic acid sequences which code for the same amino acid sequences coded by

the sequence of SEQ ID NO:1 or SEQ ID NO:3 are also included in the scope of the present invention.

Also provided, are expression vector constructs comprising the human voltage-gated calcium channel α2δ-2 isoform encoding nucleic acid molecules (SEQ ID NO:1 and SEQ ID NO:3) operably linked to a promoter. Host cells transformed or transfected with the expression vector are also within the scope of the invention.

$ The nucleic acid molecules detailed herein will find use in therapeutics or in diagnostic applications, e.g., as probes used for detecting genomic sequences comprising sequences substantially identical to those detailed herein or for detecting sequences encoding one of the novel isoforms detailed herein in a biological sample. Importantly, the presence of the α2δ2-a (splice variant#1) or α2δ2-b (splice variant #2) splice variant transcript or the level of the splice variant transcript may be indicative of a multitude of diseases, disorders and various pathological conditions typically in connection with the diseases mediated by aberrant calcium currents specified above, as well as normal conditions. As well, the ratio of the level of the transcripts of any one of the splice variants of the invention may also be compared to that of the transcripts of the reference α2δ-2 sequences from which it has been varied or compared to the level of other transcripts, and said ratio may be indicative of a multitude of diseases, disorders and various pathological and normal conditions as described above.

In another aspect, the invention provides a protein or polypeptide comprising an amino acid sequence encoded by any of the above nucleic acid sequences, termed herein "α2δ-2 splice variant product" or "splice variants" "protein isoform" or grammatical equivalents thereof. In one embodiment, the polypeptide corresponding to α2δ2-a comprises the amino acid sequence of SEQ ID NO:2. In another embodiment the polypeptide corresponding to α2δ2–b comprises the amino acid sequence of SEQ ID NO:4. Fragments of the above amino acid sequences of sufficient length coded by the above fragments of the nucleic acid sequences, as well as homologues of the above amino acid sequences in which one or more of the amino acid residues has been substituted (by conservative or non-conservative substitution) added, deleted, or chemically modified are also within the scope of the invention.

The deletions, insertions and modifications should be in regions, or adjacent to regions, wherein the splice variants differs from the reference sequence, but maintains its ability to regulate voltage-gated calcium influx. Applicants appreciate that a skilled artisan will be able to modify the splice variants or fragments thereof by addition, deletions or substitutions of amino acids (derivative product/polypeptide). Consequently, homologues of the α2δ2–a splice variants which are derivated from the reference α2δ2–a splice variant e.g., α2δ2–a (SEQ ID NO:2) or α2δ2–b (SEQ ID NO:4) by changes (deletion, addition, substitution) are also a part of

the present invention, wherein said derivatized protein is functionally equivalent to the splice variants detailed herein, i.e., ability to modulate voltage-gated calcium influx etc. In general, if the α2δ-2 splice variant is distinguished from the reference α2δ2–a or α2δ2–b sequence by some sort of physiological activity, then the homolog is distinguished from the reference α2δ-2 sequence in essentially the same manner.

Another aspect of the invention features a dominant negative human voltage-gated calcium channel corresponding to any one or both of the isoforms provided by the invention. The dominant negative polypeptide inhibits the function of the calcium channel.

Use of the foregoing compositions in the preparation of a medicament, and particularly in the preparation of a medicament for the treatment of CNS disorder including epilepsy, neuropathic pain), or a condition which results from excessive or insufficient voltage regulated calcium influx are also a part of the present invention.

The invention also provides anti-splice variant product antibodies, namely antibodies directed against the splice variants of the invention, which specifically bind to said splice variant product(s). These antibodies are useful both for diagnostic and therapeutic purposes.

Consequently, an aspect of the invention features antibodies termed "distinguishing antibodies" which are directed solely to the amino acid sequences which distinguishes the α2δ2-a or α2δ2-b splice variant from the reference α2δ-2 amino acid sequence from which the splice variants have been varied by alternative splicing. For example, these antibodies may be directed to alternative regions containing inserted new amino acid sequences. The regions may also be regions wherein a new sequence was obtained due to splicing. The distinguishing antibodies may be used for detection purposes, i.e. to detect individuals, tissue, conditions (both pathological or physiological) wherein at least one of the herein disclosed α2δ-2 isoform sequences or reference sequence are low or high (as compared to a normal control). The antibodies may also be used to distinguish conditions where the level, or ratio of the α2δ-2 splice variants, e.g., α2δ2-a or α2δ2-b to reference α2δ-2 sequence or the ratio of one splice variant (SEQ ID NO:2) to the other splice variant (SEQ ID NO:4) or other splice variants have been varied. The distinguishing antibodies may also be used for therapeutic purposes, i.e., to neutralize only the α2δ2-a subunit or the α2δ2-b subunit or only the product of the reference α2δ-2 sequence, as the case may be, without neutralizing the other.

The present invention still further provides pharmaceutical compositions comprising, as an active ingredient, said nucleic acid molecules, said expression vectors, or said protein/polypeptide or anti-splice variant product antibodies will find use in the treatment of diseases and pathological conditions where a therapeutically beneficial effect may be achieved

by neutralizing the splice variants of the invention (either at the transcript or product level) or decreasing the amount of the either of the splice variants of the invention or blocking its binding to its ligand (calcium etc) or the molecule it effects (G-protein), for example, by the neutralizing effect of the antibodies, or by decreasing the effect of the antisense mRNA in decreasing expression level of the $\alpha 2\delta$-2 subunit isoform of the invention. Typically, these diseases are of neurologic or psychiatric origin, e.g., neurodegenerative diseases, epilepsy, anxiety, schizophrenia, manic depression, depression, delirium, or for the treatment of cancer etc. In general, these are diseases wherein $\alpha 2\delta$ or other auxiliary subunit proteins of the calcium channel plays a role in the etiology of the disease, i.e. aberrant (excessive or insufficient voltage regulated calcium influx) cause or are a result of the disease.

Methods for treating subjects suffering from or at risk of being afflicted with a pathology/disease characterized by aberrant voltage regulated calcium influx are also embraced by the invention. The disease status can be characterized as aberrant – excessive or insufficient voltage regulated calcium influx relative to normal.

Thus, an aspect of the invention features a method for selectively treating a subject having a condition characterized by aberrant brain neuronal calcium current. The method features administering to a subject in need of such treatment a pharmacological agent which is selective for a human voltage-gated calcium channel $\alpha 2\delta$-2 subunit isoform, e.g., SEQ ID NO:2 or 4 , in an amount effective to normalize the aberrant neuronal calcium current. By "aberrant" is meant a level of calcium current (calcium influx) which is outside of a normal range as understood in the medical arts. Normalize means that the calcium current is brought within the normal range.

Yet another aspect of the invention features methods for (i) detecting the level of the transcript (mRNA) of said $\alpha 2\delta 2$-a or $\alpha 2\delta 2$-b splice variants products (SEQ ID NO:2 or SEQ ID NO:4, including fragments thereof) in a body fluid sample, or in a specific tissue sample, for example by use of probes comprising all or parts of the nucleotide sequences disclosed herein; (ii) detecting levels of expression of said splice variant product in tissue, e.g. by the use of antibodies capable of specifically reacting with the gene products of the nucleotide sequences of the invention or biologically equivalent fragments thereof. Detection of the level of the expression of the splice variant products of the invention in particular as compared to that of the reference sequence from which it was varied or compared to other splice variant sequences all varied from the same reference sequence may be indicative of a plurality of physiological or pathological conditions. Quantifying normal levels of the target gene or its encoded gene product are well known to a skilled artisan.

The method, according to this latter aspect, for detecting a nucleic acid sequence which encodes at least one of the human voltage-gated calcium channel $\alpha_2\delta$-2 subunit isoforms of the invention in a biological sample, comprises the steps of:

(a) providing a probe comprising at least one of the nucleic acid sequences defined above;

(b) contacting the biological sample with said probe under conditions allowing hybridization of nucleic acid sequences thereby enabling formation of hybridization complexes;

(c) detecting hybridization complexes, wherein the presence of the complex indicates the presence of nucleic acid sequence encoding the $\alpha_2\delta$-2 subunit isoform in the biological sample.

The method as described above is qualitative, i.e. indicates whether the transcript is present in or absent from the sample. The method can also be quantitative, by determining the level of hybridization complexes and then calibrating said levels to determining levels of transcripts of the desired splice variant in the sample. Both qualitative and quantitative determination methods can be used for diagnostic, prognostic and therapy planning purposes.

The nucleic acid sequence used in the above method may be a DNA sequence, an RNA sequence, etc; it may be a coding or a sequence or a sequence complementary thereto (for respective detection of RNA transcripts or coding-DNA sequences). By quantization of the level of hybridization complexes and calibrating the quantified results it is possible also to detect the level of the transcript in the sample.

Methods for detecting mutations in the region coding for any one or both of the $\alpha_2\delta$-2 isoform products are also provided, which may be methods carried-out in a binary fashion, namely merely detecting whether there is any mismatches between the normal splice variant nucleic acid sequence of the invention and the one present in the sample, or carried-out by specifically detecting the nature and location of the mutation.

The present invention also concerns a method for detecting a human voltage-gated calcium channel $\alpha_2\delta$-2 subunit isoform of the invention in a biological sample, comprising the steps of:

(a) contacting said biological sample with a detectable antibody of the invention, thereby forming an antibody-antigen complex; and

(b) detecting said antibody-antigen complex

wherein the presence of said antibody-antigen complex correlates with the presence of the $\alpha_2\delta$-2 isoform in the biological sample.

As indicated above, the method can be quantitized to determine the level or the amount of the α2δ-2 subunit isoform (SEQ ID NO:2 or 4 or equivalents fragments thereof) in the sample, alone or in comparison to the level of the reference α2δ-2 subunit amino acid sequence from which it was varied, and qualitative and quantitative results may be used for diagnostic, prognostic and therapy planning purposes.

Another aspect of the invention details a method for inhibiting human voltage-gated calcium channel α2δ-2 subunit isoform activity in a mammalian cell. The method proposes contacting the mammalian cell with an amount of a human voltage-gated calcium channel α2δ-2 subunit isoform inhibitor sufficient to inhibit calcium influx in the mammalian cell. Preferably the inhibitor is selected from the group consisting of a peptide or an antibody which selectively binds the respective α2δ-2 subunit isoform, an antisense nucleic acid which binds a nucleic acid encoding the subject isoform, and a dominant negative human voltage-gated calcium channel α2δ-2 subunit isoform.

Yet another aspect of the invention details a method for treating a subject suspected of suffering from a α2δ-2 mediated disorder – epilepsy, stroke, pain (e.g., neuropathic pain). The method details administering to a subject in need of such treatment an inhibitor of the human voltage-gated calcium channel α2δ-2 subunit polypeptide isoform in an amount effective to inhibit voltage regulated calcium influx. In another embodiment of the foregoing methods, the inhibitor is administered prophylactically to a subject at risk of having a stroke.

Utilizing a similar method, methods of increasing human voltage-gated calcium channel α2δ-2 subunit isoform expression in a cell are also provided. In certain embodiments, the cell may further be contacted with one or more human auxiliary voltage-gated calcium channel non-α2δ-2 subunits, such as a β subunit, or nucleic acid molecules encoding such non-α2δ-2 subunits.

The invention further features a method for identifying a candidate pharmacological agent useful in the treatment of diseases associated with increased or decreased voltage regulated calcium influx mediated by a human voltage-gated calcium channel α2δ-2 subunit isoform of the invention. A cell comprising a human voltage-gated calcium channel isoform of the invention is loaded with a calcium-sensitive compound which is detectable in the presence of calcium. The cell is contacted with a candidate pharmacological agent under conditions which, in the absence of the candidate pharmacological agent, cause a first amount of voltage regulated calcium influx into the cell. A test amount of voltage regulated calcium influx then is determined. For example, in a preferred embodiment, fluorescence of a calcium-sensitive compound then is detected as a measure of the voltage regulated calcium influx. If the test amount of voltage regulated calcium influx is less than the first amount, then the candidate

pharmacological agent is a lead compound for a pharmacological agent which reduces voltage regulated calcium influx. If the test amount of voltage regulated calcium influx is greater than the first amount, then the candidate pharmacological agent is a lead compound for a pharmacological agent which increases voltage regulated calcium influx.

In yet another aspect, the invention features an agent which selectively binds the human voltage-gated calcium channel $\alpha 2\delta$-2 subunit isoforms or a nucleic acid that encodes the subject isoform. By "selectively binds" it is meant that the agent binds the human voltage-gated calcium channel $\alpha 2\delta$-2 subunit isoform or nucleic acid encoding said isoform, or any fragment thereof to a greater extent than the agent binds other human $\alpha 2\delta$-2 subunit isoforms, and preferably does not bind other $\alpha 2\delta$-2 subunit isoforms. The agent may be a monoclonal antibody, a polyclonal antibody, or an antibody fragment selected from the group consisting of a Fab fragment, a F(ab)2 fragment and a fragment including a CDR3 region. In another embodiment, the agent is an antisense nucleic acid which selectively binds to a nucleic acid encoding at least one the $\alpha 2\delta$-2 subunit isoforms disclosed herein. It is preferred that the foregoing agents are inhibitors (antagonists) or agonists of the calcium channel activity of the subject $\alpha 2\delta$-2 subunit isoform. In other embodiments one may use the nucleotide sequence of SEQ ID NO:3 or the amino acid sequence of SEQ ID NO:4.

In another aspect of the invention, methods for identifying compounds which selectively or preferentially bind a human voltage-gated calcium channel $\alpha 2\delta$-2 subunit isoform of the invention are provided. In one embodiment, the method proposes providing (i) a test cell preparation which expresses the subject $\alpha 2\delta$-2 subunit isoform of the invention, e.g., $\alpha 2\delta 2$-a subunit, and providing a control cell preparation wherein said cell expresses a human voltage-gated calcium channel subunit polypeptide other than the isoform in the test preparation, e.g., $\alpha 1$b or c isoform, (ii) contacting each preparation with a compound, and (iii) the determining binding of the compound to the test cell and said control cell preparation, wherein if the compound that binds the test cell preparation but does not bind the control cell preparation is considered a candidate compound which selectively binds the subject $\alpha 2\delta 2$-a subunit isoform of the invention. Likewise, if the test compound binds the test cell preparation in an amount greater than the control cell preparation, it is a compound which preferentially binds the subject $\alpha 2\delta 2$-a subunit isoform of the invention, e.g., SEQ ID NO:2.

By yet another aspect the invention also provides a method for identifying candidate compounds capable of binding to a human voltage-gated calcium channel $\alpha 2\delta$-2 subunit isoform of the invention and modulating its activity (being either agonists or antagonists). The method includes:

(i) providing a protein or polypeptide comprising an amino acid sequence substantially as depicted in any one of SEQ ID NO:2 and SEQ ID NO:4, or a fragment of such a sequence;

(ii) contacting a candidate compound with said amino acid sequence;

(iii) measuring the physiological effect of said candidate compound on the activity of the amino acid sequences and selecting those compounds which show a significant effect on said physiological activity.

Also provided is a diagnostic method for predicting an oncogenic potential of a sample of cells, comprising:

(a) determining, in the sample, levels of expression of a gene product expressed from a nucleotide sequence of SEQ ID NO:1 or 3 or a sequence which hybridizes to one of the above sequences or its complement.

Likewise, another aspect of the invention discloses a method for following the progress of a therapeutic regiment designed to alleviate a condition characterized by abnormal or aberrant expression of a gene product expressed from the isolated nucleic acid molecule having a sequence of nucleotides as set forth in SEQ ID NO:1 or 3 comprising:

(a) assaying a sample from a subject to determine level of a parameter selected from the group consisting of (i) a polypeptide encoded by the nucleotide sequence of SEQ ID NO:1 or 3 and (ii) a polynucleotide encoding the amino acid sequence of SEQ ID NO:2 or 4, at a first time point;

(b) assaying the level of the parameter selected in (a) at a second time point and

(c) comparing said level at said second time point to the level determined in (a) as a determination of effect of said therapeutic regimé.

An alternative embodiment provides a method for determining regression, progression or onset of a pathological disorder characterized by an aberrant level or activity of a splice variant of the invention or aberrant level of voltage regulated calcium influx comprising incubating a sample obtained from a patient with said disorder with a complimentary nucleic acid hybridization probe having a sequence of nucleotides that are substantially homologous to those of SEQ ID NO:1 or 3 and determining binding between said probe and any complimentary mRNA that may be present in said sample as determinative of the regression, progression or onset of said pathological disorder in said patient.

Compounds identified by any of the herein disclosed methods are also within the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

SEQ ID NO:1 depicts the nucleic acid sequence of a splice variant of the invention ($\alpha_2\delta 2$-a).

SEQ ID NO:2 depicts the deduced amino acid sequence of $\alpha_2\delta 2$-a.

SEQ ID NO:3 depicts the nucleic acid sequence of a splice variant of the invention $\alpha_2\delta 2$-b.

SEQ ID NO:4 depicts the deduced amino acid sequence of $\alpha_2\delta 2$-b.

Figure 1 depicts the data showing binding of labeled gabapentin to each of the herein disclosed splice variants of the invention.

DETAILED DESCRIPTION OF THE INVENTION:

Before the present proteins, nucleotide sequences, and methods are described, it is to be understood that the present invention is not limited to the particular methodologies, protocols, cell lines, vectors, and reagents described, as these may vary. It is also understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not to limit the scope of the present invention.

The singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

All technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention pertains. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of protein chemistry and biochemistry, molecular biology, microbiology and recombinant DNA technology, which are within the skill of the art. Such techniques are explained fully in the literature.

Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials, and methods are now described. All patents, patent applications, and publications mentioned herein, whether supra or infra, are each incorporated by reference in its entirety.

Glossary

In the following description and claims use will be made, at times, with a variety of terms, and the meaning of such terms as they should be construed in accordance with the invention is as follows:

A "gene" refers to a nucleic acid molecule whose nucleotide sequence codes for a polypeptide molecule. Genes may be uninterrupted sequences of nucleotides or they may include such intervening segments as introns, promoter regions, splicing sites and repetitive sequences. A gene can be either RNA or DNA. A preferred gene is one that encodes the invention protein.

In defining nucleic acid sequences, all subject nucleic acid sequences capable of encoding "substantially similar amino acid sequences" are considered substantially similar or are considered as comprising substantially identical sequences of nucleotides to the reference nucleic acid sequence, i.e., $\alpha 2\delta 2$-a encoding SEQ ID NO:1.

"Homologues of variants" amino acid sequences of variants in which one or more amino acids has been added, deleted or replaced. The addition, deletion or replacement should be in regions or adjacent to regions where the $\alpha 2\delta 2$-a and $\alpha 2\delta 2$-b variants differs from the original $\alpha 2\delta$-2 sequence.

"Conservative substitution" refers to the substitution of an amino acid in one class by an amino acid of the same class, where a class is defined by common physicochemical amino acid side chain properties and high substitution frequencies in homologous proteins found in nature, as determined, for example, by a standard Dayhoff frequency exchange matrix or BLOSUM matrix. [Six general classes of amino acid side chains have been categorized and include: Class I (Cys); Class II (Ser, Thr, Pro, Ala, Gly); Class III (Asn, Asp, Gln, Glu); Class IV (His, Arg, Lys); Class V (Ile, Leu, Val, Met); and Class VI (Phe, Tyr, Trp). For example, substitution of an Asp for another class III residue such as Asn, Gln, or Glu, is a conservative substitution.

"Non-conservative substitution" refers to the substitution of an amino acid in one class with an amino acid from another class; for example, substitution of an Ala, a class II residue, with a class III residue such as Asp, Asn, Glu, or Gln.

As used herein "human N-type calcium channel $\alpha 2\delta 2$ subunit activity" refers to an ability of a molecule to modulate voltage regulated calcium influx. A molecule which inhibits human voltage-gated calcium channel $\alpha 2\delta 2$ subunit activity (an antagonist) is one which inhibits voltage regulated calcium influx via this calcium channel and a molecule which increases human voltage-gated calcium channel $\alpha 2\delta 2$ subunit activity (an agonist) is one which increases voltage regulated calcium influx via this calcium channel. Changes in human voltage-

gated calcium channel α2δ2-a subunit activity can be measured by changes in voltage regulated calcium influx by in vitro assays such as those disclosed herein, including patch-clamp assays and assays employing calcium sensitive fluorescent compounds such as fura-2.

"Agonist" refers to a molecule which, when bound to α2δ2-a or α2δ2-b, or is within proximity of α2δ2-a or α2δ2-b, modulates the activity of α2δ2-a or α2δ-2 by increasing or prolonging the duration of the effect of α2δ2-a or α2δ2-b. Agonists can include proteins, nucleic acid molecules, carbohydrates, organic compounds, inorganic compounds, or any other molecules which modulate the effect of α2δ2-a or α2δ2-b.

"Antagonist" refers to a molecule which, when bound to α2δ2-a or α2δ2-b or within close proximity, decreases the amount or the duration of the biological or immunological activity of α2δ2-a or α2δ2-b. Antagonists may include proteins, nucleic acid molecules, carbohydrates, antibodies, organic compounds, inorganic compounds, or any other molecules which exert an effect on α2δ2-a or α2δ2-b activity.

"Deletion" is a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

"Insertion" or "addition"--is that change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring sequence.

"Substitution" replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively. As regards amino acid sequences the substitution may be conservative or non- conservative.

"Antibody" refers to IgG, IgM, IgD, IgA, and IgG antibody. The definition includes polyclonal antibodies or monoclonal antibodies. This term refers to whole antibodies or fragments of the antibodies comprising the antigen-binding domain of the anti-variant product antibodies, e.g. antibodies without the Fc portion, single chain antibodies, fragments consisting of essentially only the variable, antigen-binding domain of the antibody, etc.

"Antibody" can be an intact molecule or fragments thereof, such as Fab, F(ab)2, and Fv fragments, which are capable of binding an epitopic determinant. The antibody can be polyclonal, monoclonal, or recombinantly produced.

Nucleotide sequence "similarity" is a measure of the degree to which two polynucleotide sequences have identical nucleotide bases at corresponding positions in their sequence when optimally aligned (with appropriate nucleotide insertions or deletions). Sequence similarity or percent similarity can be determined, for example, by comparing sequence information using sequence analysis software such as the GAP computer program, version 6.0, available from the University of Wisconsin Genetics Computer Group (UWGCG).

The GAP program utilizes the alignment method of Needleman and Wunsch (J. Mol. Biol. 48:443, 1970), as revised by Smith and Waterman (Adv. Appl. Math. 2:482, 1981).

The phrases "percent identity" and "% identity" refers to the percentage of sequence similarity found by a comparison or alignment of two or more amino acid or nucleic acid sequences. Percent identity can be determined by a direct comparison of the sequence information between two molecules by aligning the sequences, counting the exact number of matches between the two aligned sequences, dividing by the length of the shorter sequence, and multiplying the result by 100. "Identity" can be readily calculated by known methods. Readily available computer programs can be used to aid in the analysis, such as ALIGN, Dayhoff, M. O. in Atlas of Protein Sequence and Structure M. O. Dayhoff ed., 5 Suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., which adapts the local homology algorithm of Smith and Waterman (1981) Advances in Appl. Math. 2:482-489, for peptide analysis. Programs for determining nucleotide sequence identity are available in the Wisconsin Sequence Analysis Package, Version 8 (Genetics Computer Group, Madison, Wis.) for example, the BLAST, BESTFIT, FASTA, and GAP programs, which also rely on the Smith and Waterman algorithm. These programs are readily utilized with the default parameters recommended by the manufacturer and described in the Wisconsin Sequence Analysis Package referred to above. Other programs for calculating identity or similarity between sequences are known in the art.

The well known Smith Waterman algorithm may also be used to determine identity.

Parameters for polypeptide sequence comparison include the following:

1) Algorithm: Needleman and Wunsch, J. Mol. Biol. 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992).

Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison Wis. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Parameters for polynucleotide comparison include the following:

1) Algorithm: Needleman and Wunsch, J. Mol. Biol. 48: 443-453 (1970)

Comparison matrix: matches=+10, mismatch=0

Gap Penalty: 50

Gap Length Penalty: 3

Available as: The "gap" program from Genetics Computer Group, Madison Wis. These are the default parameters for nucleic acid comparisons.

A preferred meaning for "identity" for polynucleotides and polypeptides, as the case may be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, wherein the polynucleotide sequence may be identical to the reference sequence of SEQ ID NO:1 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein the alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein the alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein the number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from the total number of nucleotides in SEQ ID NO:1, or:

Nn Xn - (Xn Y),

wherein Nn is the number of nucleotide alterations, Xn is the total number of nucleotides in SEQ ID NO:1, Y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and is the symbol for the multiplication operator, and wherein any non-integer product of Xn and Y is rounded down to the nearest integer prior to subtracting it from Xn alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

(2) Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50,60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein the polypeptide sequence may be identical to the reference sequence of SEQ ID NO:2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein the alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein the alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino

acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein the number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from the total number of amino acids in SEQ ID NO:2, or:

$$Na = Xa - (Xa\ Y),$$

wherein Na is the number of amino acid alterations, Xa is the total number of amino acids in SEQ ID NO:2, Y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and is the symbol for the multiplication operator, and wherein any non-integer product of Xa and Y is rounded down to the nearest integer prior to subtracting it from Xa.

For example, a designated amino acid percent identity of 70% refers to sequences or subsequences that have at least about 70% amino acid identity when aligned for maximum correspondence over a comparison window as measured using one of the sequence comparison algorithms disclosed herein and well known to a skilled artisan or by manual alignment and visual inspection. It is recognized, however, that proteins (and DNA or mRNA encoding such proteins) containing less than the above-described level of homology arising as splice variants or that are modified by conservative amino acid substitutions (or substitution of degenerate codons) are contemplated to be within the scope of the present invention.

"Optimal alignment"--is defined as an alignment giving the highest percent identity score. Such alignment can be performed using a variety of commercially available sequence analysis programs, such as the local alignment program LALIGN using a ktup of 1, default parameters and the default PAM. A preferred alignment is the one performed using the CLUSTAL-W program from MacVector (TM), operated with an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM similarity matrix. If a gap needs to be inserted into a first sequence to optimally align it with a second sequence, the percent identity is calculated using only the residues that are paired with a corresponding amino acid residue (i.e., the calculation does not consider residues in the second sequences that are in the "gap" of the first sequence). In case of alignments of known gene sequences with that of the new variant, the optimal alignment invariably included aligning the identical parts of both sequences together, then keeping apart and unaligned the sections of the sequences that differ one from the other.

"Having at least 90% identity" with respect to two amino acid or nucleic acid sequence sequences, refers to the percentage of residues that are identical in the two sequences when the sequences are optimally aligned. Thus, 90% amino acid sequence identity means that 90% of the amino acids in two or more optimally aligned polypeptide sequences are identical,

however this definition explicitly excludes sequences which are 100% identical with the original sequence from which the variant of the invention was varied.

"Treating a disease" refers to administering a therapeutic substance effective to ameliorate symptoms associated with a disease, to lessen the severity or cure the disease, or to prevent the disease from occurring.

"Detection" refers to a method of detection of a disease, disorder, pathological or normal condition. This term may refer to detection of a predisposition to a disease as well as for establishing the prognosis of the patient by determining the severity of the disease.

"Original α2δ-2 sequence" or "reference sequence" is used interchangeably to refer to the amino acid or nucleic acid sequence from which the α2δ-2 variants(s) of the invention have been varied as a result of alternative slicing. The original sequence (reference sequence) is the sequence of the human voltage-gated calcium channel and the sequence is published as GenBank Accession # AF042792. See also, Gao et al, *supra.*

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification can be carried out using polymerase chain reaction (PCR) technologies or other methods well known in the art.

The term "antisense" or "antisense oligonucleotides" refers to short synthetic nucleotide sequences formulated to be complementary to a portion of a specific gene or mRNA. They function by hybridizing to complementary sequences, resulting in the selective arrest of expression of the complementary gene or mRNA. In addition to the use of antisense oligonucleotides as therapeutic agents due to their ability to block expression of a specific target protein, they also provide a useful tool for exploring regulation of the expression of a gene of interest and in tissue culture (see Rothenberg, M., et al., Natl. Cancer Inst., 81:1539-1544, 1989).

The term "expression" as used herein intends both transcriptional and translational processes, i.e., the production of messenger RNA and/or the production of protein therefrom.

The term "modulate" refers to a change in the activity of the variants detailed herein. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of α2δ2-a or α2δ2-b. The ability to modulate the activity of α2δ2-a or α2δ2-b can be exploited in assays to screen for organic, inorganic, or biological compounds which affect the above properties of α2δ2-a or α2δ2-b.

A "reporter gene" is a gene that, upon expression, confers a phenotype on a cell expressing the reporter gene, such that the cell can be identified under appropriate conditions. For example, the reporter gene may produce a polypeptide product that can be easily detected or

measured in a routine assay. Suitable reporter genes known in the art which confer this characteristic include those that encode chloramphenicol acetyl transferase (CAT activity), β-galactosidase, luciferase, alkaline phosphatase, human growth hormone, fluorescent proteins, such as green fluorescent protein (GFP), and others. Indeed, any gene that encodes a protein or enzyme that can readily be measured, for example, by an immunoassay such as an enzyme-linked immunosorbent assay (ELISA) or by the enzymatic conversion of a substrate into a detectable product, and that is substantially not expressed in the host cells (specific expression with no background) can be used as a reporter gene to test for promoter activity. Other reporter genes for use herein include genes that allow selection of cells based on their ability to thrive in the presence or absence of a chemical or other agent that inhibits an essential cell function. Suitable markers, therefore, include genes coding for proteins which confer drug resistance or sensitivity thereto, or change the antigenic characteristics of those cells expressing the reporter gene when the cells are grown in an appropriate selective medium. For example, reporter genes include: cytotoxic and drug resistance markers, whereby cells are selected by their ability to grow on media containing one or more of the cytotoxins or drugs; auxotrophic markers by which cells are selected by their ability to grow on defined media with or without particular nutrients or supplements; and metabolic markers by which cells are selected for, e.g., their ability to grow on defined media containing the appropriate sugar as the sole carbon source. These and other reporter genes are well known in the art.

A "change in the level of reporter gene product" is shown by comparing expression levels of the reporter gene product in a cell exposed to a candidate compound relative to the levels of reporter gene product expressed in a cell that is not exposed to the test compound and/or to a cell that is exposed to a control compound. The change in level can be determined quantitatively for example, by measurement using a spectrophotometer, spectrofluorometer, luminometer, and the like, and will generally represent a statistically significant increase or decrease in the level from background. However, such a change may also be noted without quantitative measurement simply by, e.g., visualization, such as when the reporter gene is one that confers the ability on cells to form colored colonies on chromogenic substrates.

"Subject" means mammals and non-mammals. Mammals means any member of the Mammalia class including, but not limited to, humans, non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, and swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice, and guinea pigs; and the like. Examples of non-mammals include, but are not limited to, birds, and the like. The term "subject" does not denote a particular age or sex.

The term "substantially purified," when referring to a polypeptide, indicates that the polypeptide is present in the substantial absence of other similar biological macromolecules.

The term "transformed" refers to any known method for the insertion of foreign DNA or RNA sequences into a host prokaryotic cell. The term "transfected" refers to any known method for the insertion of foreign DNA or RNA sequences into a host eukaryotic cell. Such transformed or transfected cells include stably transformed or transfected cells in which the inserted DNA is rendered capable of replication in the host cell. They also include transiently expressing cells which express the inserted DNA or RNA for limited periods of time. The transformation or transfection procedure depends on the host cell being transformed. It can include packaging the polynucleotide in a virus as well as direct uptake of the polynucleotide, such as, for example, lipofection or microinjection. Transformation and transfection can result in incorporation of the inserted DNA into the genome of the host cell or the maintenance of the inserted DNA within the host cell in plasmid form. Methods of transformation are well known in the art and include, but are not limited to, viral infection, electroporation, lipofection, and calcium phosphate mediated direct uptake.

"Treating" or "treatment" of a disease state includes: 1) preventing the disease state, i.e. causing the clinical symptoms of the disease state not to develop in a subject that may be exposed to or predisposed to the disease state, but does not yet experience or display symptoms of the disease state; 2) inhibiting the disease state, i.e., arresting the development of the disease state or its clinical symptoms; 3) or relieving the disease state, i.e., causing temporary or permanent regression of the disease state or its clinical symptoms.

A "variant" of $\alpha_2\delta$2-a or $\alpha_2\delta$2-b polypeptides refers to an amino acid sequence that is altered by one or more amino acid residues. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine.) More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan.) Analogous minor variations may also include amino acid deletion or insertions, or both. Guidance in determining which amino acid variations may be substituted, inserted, or deleted without abolishing biological function may be found using programs well known in the art, for example, LASERGENE software (DNASTAR).

The Invention

The present invention is based on the discovery of two novel human voltage-gated calcium channel $\alpha_2\delta$-2 subunit variants -$\alpha_2\delta$2-a and $\alpha_2\delta$2-b, the polynucleotides encoding each of $\alpha_2\delta$2-a and $\alpha_2\delta$2-b, as well as therapeutics relating thereto. The invention also embraces isolated functionally equivalent variants, useful analogs and fragments of the

foregoing nucleic acid molecules and polypeptides; complements of the foregoing nucleic acid molecules; and molecules which selectively bind the foregoing nucleic acid molecules, and the use of the above for screening compounds useful in the treatment or prevention of a dysfunctional calcium channel, e.g., dysfunctional voltage-gated voltage regulated calcium influx mediated by a $\alpha 2\delta$-2 calcium subunit.

The novel splice variants of the invention may have the same physiological activity as the reference $\alpha 2\delta$-2 from which they are varied (although perhaps at a different level) considering that they share 99.9% and 97.6 % amino acid sequence primary sequence identity with the reference sequence. However, the novel splice variants may differ from the reference sequence by their agonist-dependent or agonist independent activity. In addition, either one or both of the novel isoforms of the invention may differ from the reference sequence in their affinity to its native ligand/binding partner (calcium) or affinity to various agonist and antagonists coupling efficiency to G-protein which they can activate, or alternatively may have no activity at all and this may lead to various diseases or pathological conditions.

Both in the case the $\alpha 2\delta$-2 subunit isoform(s) of the invention has the same activity as the reference sequence as well as the case it has the opposite activity from that of the reference sequence, it may differ from the reference sequence in its stability, its clearance rate, its rate of degradation, its tissue and cellular distribution, its ligand specification, its temporal expression pattern, its pattern and mechanism of up and down regulation and in other biological properties not necessarily connected to activity.

In addition to therapeutic purposes, the novel $\alpha 2\delta$-2 subunit isoforms may be very useful for detection purposes, i.e. its presence or level may be indicative of a disease, disorder, pathological or normal condition involving an $\alpha 2\delta$-2 isoform. Representative diseases include disorders of neurologic or psychiatric origin such as, for example, neurodegenerative disease, epilepsy, anxiety, schizophrenia, manic depression, depression, delirium, the treatment of cancer. Alternatively, the ratio between the level of either or both of the $\alpha 2\delta$-2 isoforms and the level of the reference peptide from which it has been varied, or the level of one $\alpha 2\delta$-2 isoform to the other may be indicative to such a disease, disorder, pathological or normal condition, for example, any one of the diseases specified above.

For representative purposes, it is possible to establish differential expression of the splice variant(s) in various tissues as compared to the reference $\alpha 2\delta$-2 subunit sequence. The $\alpha 2\delta$-2 isoforms may be expressed mainly in one tissue, while the reference $\alpha 2\delta$-2 subunit sequence from which it has been varied may be expressed mainly in another tissue. Understanding of the distribution of the splice variants in various tissues may be helpful, for example, in understanding the physiological function of the genes as well as helping in targeting

pharmaceuticals or developing pharmaceuticals. In addition, deviation from normal distribution may be indicative of any of the above diseases.

The study of the α2δ-2 isoforms may also be helpful to distinguish various stages in the life cycles of the cells which may also be helpful for development of pharmaceuticals for various pathological conditions in which cell cycles is abnormal. Such abnormal can lead either to various developmental problems concerning the nervous system or alternatively can lead to various types of cancer, or to diseases involving the nervous system, both neuronal and psychiatric origin or any one of the diseases specified above.

The detection may by determination of the presence or the level of expression of the α2δ-2 isoforms within a specific cell population, and comparing the presence or level between various cell types in a tissue, between different tissues and between individuals.

The α2δ-2 isoform molecules of the present invention were isolated as detailed in Example 1.

I. Nucleic Acid Molecules of the Invention; Vectors; and Recombinant Host Cells of the Invention.

A. In a first aspect, the invention provides isolated nucleic acid molecules that encode a functional auxiliary subunit of a human voltage-gated calcium channel. Each of the novel isoform sequences codes for a novel, naturally occurring, alternative splice variant of the native and known calcium channel α2δ-2 subunit, the sequence of which as disclosed in GenBank accession # AFO42792 as well as referenced in Gao et al., J. Biol. Chem., 16; 12237-12242 (2000) (reference sequence). It should be emphasized that the novel isoform sequences are naturally occurring sequences resulting from alternative splicing of the native α2δ-2 subunit gene and not merely truncated, mutated or fragmented forms of the gene. Specifically, the invention discloses nucleic acid molecules encoding novel human isoforms of the voltage-gated calcium channel, referred to herein as the human voltage-gated calcium channel α2δ2-a subunit (α2δ2-a isoform) and α2δ2-b subunit (α2δ2-b isoform).

As used herein "human voltage-gated calcium channel α2δ2-a subunit encoding nucleic acid molecule" or "variant encoding nucleic acids" "isoform nucleic acid molecules" refers not only to an isolated nucleic acid molecule which codes for a human voltage-gated calcium channel α2δ2-a subunit isoform comprising nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3 in the case of α2δ2-b, but also nucleotide sequences having at least 90% identity to said sequences (SEQ ID NO:1 or 3) and fragments of the above sequences, which encode a biologically active voltage-gated calcium channel subunit polypeptide isoform. In this context, "biologically active," refers to a protein having structural, regulatory, or biochemical

functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic α2δ2 subunit isoform, e.g., SEQ ID NO:2, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

As well, the term "human voltage-gated calcium channel α2δ-2 subunit nucleic acid molecules" or grammatically equivalent terms thereof also includes those nucleic acid molecules which code for a human voltage-gated calcium channel α2δ-2 subunit polypeptide isoform comprising the amino acid sequences as set forth in one of SEQ ID NO:2 and 4. More, the nucleic acid molecules comprising nucleotide sequences which differ from the sequence of SEQ ID NO:1 or 3 in codon sequence due to the degeneracy of the genetic code are also within the scope of the invention. The nucleic acid molecules of the invention also include biologically active fragments of the foregoing nucleic acid molecules, provided that the fragment encodes the amino acid sequence of SEQ ID NO:2 or 4 or biologically active fragments thereof. While the specification may detail the human voltage-gated calcium channel α2δ2–1 subunit isoform and its uses, it is understood that the same applies to the human voltage-gated calcium channel α2δ2–2 subunit isoform (SEQ ID NO:4). For example, just as the human voltage-gated calcium channel α2δ2-a subunit isoform encoding nucleic acid is "isolated", so is the human voltage-gated calcium channel α2δ2-b subunit isoform encoding nucleic acid molecule.

The term "isolated", as used herein in reference to a nucleic acid molecule, means a nucleic acid sequence: (i) amplified *in vitro* by, for example, polymerase chain reaction (PCR); (ii) synthesized by, for example, chemical synthesis; (iii) recombinantly produced by cloning; or (iv) purified, as by cleavage and electrophoretic or chromatographic separation from natural contaminants, e.g., free of other nucleic acid molecules that do not encode the subject polypeptide.

The term "nucleic acid sequence" "nucleic acid molecule" or grammatical equivalents thereof is intended for ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) of genomic or synthetic origin which may be single stranded or double stranded and may represent the sense of the antisense strand, a peptide nucleic acid (PNA), or any DNA-like or RNA-like material. DNA can be either complementary DNA (cDNA) or genomic DNA. "Peptide nucleic acid" (PNA), as used herein, refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA and RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell. See, e.g., Nielsen, P. E. et al. (1993) Anticancer Drug Des. 8:53-63. In this context, "fragments" refer to those nucleic acid

molecules which, when translated, would produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain, e.g., ion channel domain, calcium influx activity etc. characteristic of the full-length polypeptide.

"Complementary" and "complementarity" refer to the natural binding of polynucleotides to other polynucleotides by base pairing. For example, the sequence "5'A-C-G-T 3'" will bind to the complementary sequence "3'T-G-C-A 5'" Complementarity between two single stranded molecules may be "partial," such that only some of the nucleic acid molecules bind, or it may be "complete," such that total complementarity exists between the single stranded molecules.

Also encompassed by the invention are polynucleotides that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in one of SEQ ID NO:1 and 3, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) Methods Enzymol. 152:399-401; Kimmel, A. R. (1987) Methods Enzymol. 152:507-511.) "Hybridization" refers to the binding of complementary strands of nucleic acid (i.e., sense:antisense strands or probe:target-DNA) to each other through hydrogen bonds, similar to the bonds that naturally occur in chromosomal DNA. Stringency levels used to hybridize a given probe with target-DNA can be readily varied by those of skill in the art. A hybridization complex may be formed in solution (conditions calculated by performing, e.g., Cot or Rot) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins, glass slides, or any other appropriate substrate to which cells or their nucleic acid molecules have been fixed.) For example, stringent salt concentration will ordinarily be less that about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C., preferably at least about 37°C., and more preferably 42°C. Varying additional parameters such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. Nucleic acid hybridization parameters may be found in references which compile such methods, e.g. Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F. M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York.

As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature (Tm) of the hybrids. Tm can be approximated by the formula:

81.5°C.-16.6(log10 [Na+])+0.41(%G+C)-600/1,

where 1 is the length of the hybrids in nucleotides. Tm decreases approximately 1°-1.5°C with every 1% decrease in sequence homology. In general, the stability of a hybrid is a function of sodium ion concentration and temperature.

Typically, the hybridization reaction is performed under conditions of lower stringency, followed by washes of varying, but higher, stringency. Reference to hybridization stringency relates to such washing conditions. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. There are other conditions, reagents, and so forth which can be used, which result in a similar degree of stringency. The skilled artisan will be familiar with such conditions, and thus they are not given here. It will be understood, however, that the skilled artisan will be able to manipulate the conditions in a manner to permit the clear identification of alleles of human voltage-gated calcium channel $\alpha_2\delta 2$-a subunit nucleic acid molecules of the invention. The skilled artisan also is familiar with the methodology for screening cells and libraries for expression of such molecules which then are routinely isolated, followed by isolation of the pertinent nucleic acid molecule and sequencing.

As used herein, the phrase "moderately stringent hybridization" refers to conditions that permit target-DNA to bind a complementary nucleic acid that has about 60% identity, preferably about 75% identity, more preferably about 85% identity to the target DNA; with greater than about 90% identity to target-DNA being especially preferred. Preferably, moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 65°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like. (See Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989).

"High stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0. 0 15 M sodium chloride/0. 00 15 M sodium citrate/0. 1 % sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50 % (v/v) formamide with 0. 1 % bovine serum albumin/0. 1 % Ficoll/O. 1 % polyvinylpyrrolidone/5OmM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium

phosphate (pH 6.8), 0.1 % sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 Jig/ml), 0. 1 % SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0. 1 x SSC containing EDTA at 55°C.

The phrase "low stringency hybridization" refers to conditions equivalent to hybridization in 10% formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at 42°C, followed by washing in 1X SSPE, 0.2% SDS, at 50°C.

The washing steps which follow hybridization can also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash step will ordinarily include temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C.

More specifically, stringent conditions, as used herein, refers, for example, to hybridization at 65°C in hybridization buffer (3.5 X SSC, 0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 0.02% Bovine Serum Albumin, 2.5 mM NaH.sub.2PO.sub.4(pH7), 0.5% SDS, 2 mM EDTA). SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH7; SDS is sodium dodecyl sulphate; and EDTA is ethylenediaminetetracetic acid. After hybridization, the membrane upon which the DNA is transferred is washed at 2 X SSC at room temperature and then at 0.1X SSC/0.1XSDS at temperatures up to 65°C.

In practice, the term "substantially the same sequence" means that DNA or RNA encoding two proteins hybridize under moderately stringent conditions and encode proteins that have the same sequence of amino acids or have changes in sequence that do not alter their structure or function.

Thus, an aspect of the invention embraces those nucleic acid molecules comprising coding sequences which are substantially the same as those set forth in SEQ ID NO:1 and which hybridize to a nucleic acid molecule comprising of SEQ ID NO:1 under moderately stringent conditions.

A "coding sequence" is a polynucleotide sequence that is transcribed into mRNA and translated into a polypeptide. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to, mRNA, cDNA, synthetic DNA, and

recombinant polynucleotide sequences. Also included is genomic DNA where the coding sequence is interrupted by introns.

Preferred nucleic acid molecules encoding the invention polypeptide(s) hybridize under moderately stringent, preferably high stringency, conditions to substantially the entire sequence, or substantial portions (i.e., typically at least 15-30 nucleotides) of the nucleic acid sequence set forth in SEQ ID NO:1 or 3.

In screening for human voltage-gated calcium channel $\alpha_2\delta$2-a subunit nucleic acid molecules, a Southern blot may be performed using the foregoing stringent conditions, together with a radioactive probe. After washing the membrane to which the DNA is finally transferred, the membrane can be placed against X-ray film to detect the radioactive signal.

The human voltage-gated calcium channel $\alpha_2\delta$2-a subunit nucleic acid molecule of the invention also include degenerate nucleic acid molecules which include alternative codons to those present in the native materials. For example, serine residues are encoded by the codons TCA, AGT, TCC, TCG, TCT and AGC. Each of the six codons is equivalent for the purposes of encoding a serine residue. Thus, it will be apparent to one of ordinary skill in the art that any of the serine-encoding nucleotide triplets may be employed to direct the protein synthesis apparatus, *in vitro* or *in vivo*, to incorporate a serine residue into an elongating human voltage-gated calcium channel $\alpha_2\delta$2-a subunit polypeptide. Similarly, nucleotide sequence triplets which encode other amino acid residues include, but are not limited to: CCA, CCC, CCG and CCT (proline codons); CGA, CGC, CGG, CGT, AGA and AGG (arginine codons); ACA, ACC, ACG and ACT (threonine codons); AAC and AAT (asparagine codons); and ATA, ATC and ATT (isoleucine codons). Other amino acid residues may be encoded similarly by multiple nucleotide sequences. Thus, the invention embraces degenerate nucleic acid molecules that differ from the biologically isolated nucleic acid molecules in codon sequence due to the degeneracy of the genetic code.

The present invention also encompasses nucleic acid molecules which differ from the nucleic acid molecule shown in SEQ ID NO:1, but which have the same phenotype. "Phenotypically similar nucleic acid molecules" are also referred to as "functionally equivalent" nucleic acid molecules.

As used herein, the phrase "functionally equivalent nucleic acid molecules" "altered nucleic acid molecules" or grammatical equivalents thereof encompasses nucleic acid molecules characterized by slight and non-consequential sequence variations that will function in substantially the same manner to produce the same protein product(s) as the nucleic acid molecules disclosed herein. These changes include those recognized by those of skill in the art as those that do not substantially alter the tertiary structure of the protein. Thus "altered" nucleic

acid sequences encoding a α2δ-2 subunit isoform, as described herein, include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide encoding the same α2δ2-a subunit isoform of SEQ ID NO:2 or a polypeptide with at least one functional characteristic of α2δ-2 subunit isoform from which it is derived.

Also provided are fragments of SEQ ID NO:1. The fragments will find use as probes in Southern blot assays to identify such nucleic acid molecules, or can be used in amplification assays such as those employing PCR. For example, smaller fragments are those comprising 12, 13, 14, 15, 16, 17, 18, 20, 22, 25, 30, 40, 50, or 75 nucleotides, and every integer therebetween and are useful e.g. as primers for nucleic acid amplification procedures – use for PCR etc. Likewise, as well known to one skilled in the art, larger probes comprising 200, 250, 300, 400 or more nucleotides are preferred for certain uses including Southern blots. Fragments of the invention also can be used to produce fusion proteins for generating antibodies, use in immunoassays, or as "immunologically active fragment" thereof. "Immunologically active fragment(s)" or "immunogens" refer to those fragments which are capable of eliciting an immune response to produce antibodies immunospecific for the splice variants/protein(s) of the invention.

A representative example includes those fragments that are capable of raising, for example, α2δ2-a subunit specific antibodies in a target immune system (e.g., murine or rabbit) or of competing with native α2δ-2 subunit for binding to α2δ2-a subunit specific antibodies, and is thus useful in immunoassays for the presence of α2δ2-a subunit peptide in a biological sample. Such immunologically active fragments typically have a minimum size of 8 to 11 consecutive amino acids. As well, it is preferable that the immunologically active fragment(s) be identical to a portion of the amino acid sequence of the human α2δ2-a subunit protein disclosed herein or a portion of the amino acid sequence from which it is derived.

Immunological methods for detecting and measuring the expression of, for example, α2δ2-a subunit peptides using either specific polyclonal or monoclonal antibodies are known in the art. Exemplary techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on α2δ2-a subunit peptides is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul Minn., Sect. IV; Coligan, J. E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York N.Y.; and Pound, J. D. (1998) Immunochemical Protocols, Humana Press, Totowa N.J.).

The foregoing nucleic acid fragments of the invention further include those sequences that can be used as antisense molecules to inhibit the expression of human voltage-gated calcium channel α2δ2-a subunit nucleic acid molecules and polypeptides, particularly for therapeutic purposes as noted below. The foregoing fragments do not necessarily produce biologically active fragments of the isoform nucleic acid molecules since these fragments do not , if translated, produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain, e.g., ion channel domain, calcium influx activity etc. characteristic of the full-length polypeptide.

An alternative embodiment features a human voltage-gated calcium channel α2δ2-a subunit isoform encoding nucleic acid, operably linked to a gene expression sequence which directs the expression of the human voltage-gated calcium channel α2δ2-a subunit within a eukaryotic or prokaryotic cell. The term "gene expression sequence" refers to any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the subject α2δ-2 subunit isoform nucleic acid to which it is operably linked. The gene expression sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, β-actin promoter and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the simian virus, papilloma virus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus, cytomegalovirus, the long terminal repeats (LTR) of Moloney murine leukemia virus and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters – these are expressed in the presence of an inducing agent. For example, the metallothionein promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

Generally, the gene expression sequence shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined human voltage-gated calcium channel α2δ2-a subunit nucleic acid. The gene expression sequences may further include enhancer sequences or upstream activator sequences as desired.

The subject α2δ2-a subunit isoform nucleic acid sequence and the gene expression sequence are "operably linked" when they are covalently linked in such a way as to place the transcription and/or translation of the subject α2δ-2 subunit isoform coding sequence under the influence or control of the gene expression sequence. In the event it is desired that a particular α2δ-2 subunit isoform sequence be translated into a functional protein, two DNA sequences are said to be operably linked if induction of a promoter in the 5' gene expression sequence results in the transcription of the target protein, vis-à-vis the desired α2δ-2 subunit isoform , e.g., human voltage-gated calcium channel α2δ2-a subunit and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the desired α2δ-2 subunit isoform sequence, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Consequently, a gene expression sequence is operably linked to a α2δ-2 subunit isoform nucleic acid molecule if the gene expression sequence is capable of effecting transcription of the gene product encoded by the desired isoform nucleic acid sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

The α2δ-2 isoform encoding nucleic acid molecules of the invention including any inhibitors of said nucleotide sequences or encoded proteins can be delivered to the eukaryotic or prokaryotic cell alone or in association with a vector. The term "vector, in its broadest sense refers to any vehicle capable of facilitating: (1) delivery of a nucleic acid molecule of the invention, to a target cell or (2) uptake of a α2δ-2 isoform nucleic acid or isoform polypeptide by a target cell. Preferably, the vectors transport the subject α2δ2 subunit isoform nucleic acid or polypeptide isoform into the target cell with reduced degradation relative to the extent of degradation that would result in the absence of the vector. Optionally, a "targeting ligand" can be attached to the vector to selectively deliver the vector to a cell which expresses on its surface the cognate receptor (e.g. a receptor, an antigen recognized by an antibody) for the targeting ligand. In this manner, the vector (containing the desired α2δ-2 subunit isoform nucleic acid or its encoded gene product can be selectively delivered to a specific cell.

In general, vectors useful in the practice of the invention can be divided into two classes: biological vectors and chemical/physical vectors. For example, biological vectors are more useful for delivery/uptake of the desired human voltage-gated calcium channel α2δ-2 isoform nucleic acid molecules to/by a target cell, whereas chemical/physical vectors are more useful for delivery/uptake of a human voltage-gated calcium channel α2δ-2 isoform subunit

nucleic acid molecules or human voltage-gated calcium channel α2δ2-a subunit proteins to/by a target cell.

Exemplary biological vectors include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the nucleic acid sequences of the invention, and free nucleic acid fragments which can be attached to the nucleic acid sequences of the invention. Viral vectors are a preferred type of biological vector and include, but are not limited to, nucleic acid sequences from the following viruses: retroviruses, such as Moloney murine leukemia virus; Harvey murine sarcoma virus; murine mammary tumor virus; Rous sarcoma virus; adenovirus; adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; and polio virus. One can readily employ other vectors not named but known in the art.

Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest, e.g., α2δ2-a subunit encoding nucleic acid molecule. Non-cytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. In general, the retroviruses are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes in vivo. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell line with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are detailed in Kriegler, M., "Gene Transfer and Expression, A Laboratory Manual, " W. H. Freeman C. O., New York (1990) and Murry, E. J. Ed. "Methods in Molecular Biology," vol. 7, Humana Press, Inc., Clifton, N.J. (1991).

Another preferred virus for certain applications is the adeno-associated virus, a double-stranded DNA virus. The adeno-associated virus can be engineered to be replication-deficient and is capable of infecting a wide range of cell types and species. Other advantages include features such as heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hemopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. As well, it has been reported that the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression. In addition,

wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, thus implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

Expression vectors comprising all the necessary elements for expression are commercially available and known to those skilled in the art. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989. Cells are genetically engineered by the introduction into the cells of heterologous DNA (RNA) encoding the desired $\alpha_2\delta$-2 polypeptide isoform or fragment or variant thereof. That heterologous DNA (RNA) is placed under operable control of transcriptional elements to permit the expression of the heterologous DNA in the host cell.

Systems suitable for mRNA expression in mammalian cells include pRc/CMV (available from Invitrogen, Carlsbad, Calif.) that contain a selectable marker such as a gene that confers G418 resistance (which facilitates the selection of stably transfected cell lines) and the human cytomegalovirus (CMV) enhancer-promoter sequences. In addition, the pCEP4 vector (Invitrogen), which contains an Epstein Barr virus (EBV) origin of replication, facilitating the maintenance of plasmid as a multicopy extrachromosomal element is very suitable for expression in primate or canine cell lines. The pEF-BOS plasmid containing the promoter of polypeptide Elongation Factor 1α, which stimulates efficiently transcription in vitro is also suitable for mRNA expression. See., e.g., Mishizuma and Nagata (Nuc. Acids Res. 18:5322, 1990), wherein the plasmid is described. See also, by and its use in transfection experiments is disclosed by, for example, Demoulin (Mol. Cell. Biol. 16:4710-4716, 1996), which details its use in transfection experiments. Other preferred expression vector include an adenovirus, described by Stratford-Perricaudet, which is defective for E1 and E3 proteins (J. Clin. Invest. 90:626-630, 1992).

In addition to biological vectors, one skilled in the art may also make use of chemical/physical vector to deliver the desired $\alpha_2\delta$-2 subunit isoform encoding nucleic acid molecule or polypeptide(s) to a target cell and facilitate uptake thereby. The term "Chemical/physical vector" refers to a natural or synthetic molecule, other than those derived from bacteriological or viral sources, capable of delivering an isolated $\alpha_2\delta$-2 subunit isoform nucleic acid or polypeptide to a cell.

An exemplary vector that falls within this class is a colloidal dispersion system. Colloidal dispersion systems include lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. Preferably, the colloidal system is a liposome, which are artificial membrane vesicles which are useful as a delivery vector in vivo or in vitro. Fraley,

et al., Trends Biochem. Sci., v. 6, p. 77, 1981 have shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0μ can encapsulate large macromolecules. RNA, DNA, and intact virions can be encapsulated within the aqueous interior and deliver biologically active forms of said cargo to cells. However, for a liposome to be an efficient nucleic acid transfer vector, one or more of the following characteristics should be present: (1) encapsulation of the nucleic acid of interest at high efficiency with retention of biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information. Reportedly, liposomes may be targeted to a particular tissue by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein. Ligands useful for targeting a liposome to a particular cell depends on the particular cell or tissue type. As well, in cases where the vector encapsulates a nucleic acid, the vector may be coupled to a nuclear targeting peptide, which will facilitate delivery of the desired α2δ-2 subunit isoform nucleic acid to the nucleus of the host cell. Liposomes are commercially available from Gibco BRL, for example, as LIPOFECTIN.TM. and LIPOFECTACE.TM., which are formed of cationic lipids such as N-[1-(2,3 dioleyloxy)-propyl]-N,N,N-trimethylammonium chloride (DOTMA) and dimethyl dioctadecylammonium bromide (DDAB). One skilled in the art is well aware of methods for making liposomes and are described in many publications. For a review of Liposomes in general, refer to Gregoriadis, G. in Trends in Biotechnology, V. 3, p. 235-241 (1985).

Other exemplary compositions that can be used to facilitate uptake by a target cell of a α2δ-2 subunit isoform nucleic acid molecules include calcium phosphate and other chemical mediators of intracellular transport, microinjection compositions, electroporation and homologous recombination compositions (e.g., for integrating the desired α2δ2 subunit isoform nucleic acid into a preselected location within a target cell chromosome).

Consequently, one skilled in the art will readily recognize that the invention also embraces the use of herein disclosed cDNA sequences in expression vectors, as well as to transfect host cells and cell lines, be these prokaryotic (e.g., E. coli), or eukaryotic (e.g., COS cells, yeast expression systems and recombinant baculovirus expression in insect cells). Especially useful are mammalian cells such as human, pig, goat, primate, etc. They may be of a wide variety of tissue types, and include primary cells and cell lines. Specifically, neuronal cells including PC 12 cells, Xenopus oocytes, bone marrow stem cells and embryonic stem cells.

Yet another aspect of the invention concerns expression kits, which enable the artisan to prepare a desired expression vector or vectors. Such expression kits include at least

separate portions of the coding sequences of the herein disclosed nucleic acid molecules. Other components may be added, as desired.

B. Peptide Nucleic Acids or "PNAs":

In yet another embodiment, the nucleic acid molecules of the invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. et al. (1996) Bioorganic & Medicinal Chemistry 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. et al. (1 996) supra; Perry-O'Keefe et al. Proc. Natl. Acad. Sci. 93: 14670-675.

Consequently, PNAs of the novel human voltage-gated calcium channel $\alpha 2\delta$-2 subunit isoform encoding nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of the nucleic acid molecules of the invention can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as "artificial restriction enzymes" when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. (1996) supra)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. et al. (1996) supra; Perry-O'Keefe supra).

In another embodiment, PNAs of the invention can be modified, (e.g., to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of the nucleic acid molecules of the invention can be generated which may combine the advantageous properties of PNA and DNA.

Such chimeras allow DNA recognition enzymes, (e.g., RNAse H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) supra). The synthesis of PNA-DNA chimeras can be performed as

described in Hyrup B. (1996) supra and Finn P. J. et al. (1996) Nucleic Acids Res. 24 (17):3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. et al. (1989) Nucleic Acid Res. 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P. J. et al. (1996) supra). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K. H. et al. (1975) Bioorganic Med. Chem. Lett. 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al. (1989) Proc. Natl. Acad. Sci. US. 86:6553-6556; Lemaitre et al. (1987) Proc. Natl. Acad. Sci. USA 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides of the invention can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al. (1988) Bio-Techniques 6:958-976) or intercalating agents. (See, e.g., Zon (1988) Pharm. Res. 5:539-549). To this end, the oligonucleotides may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

II. Substantially Pure $\alpha 2\delta 2$-a and $\alpha 2\delta 2$-b Polypeptides

Polypeptides – The present invention further provides substantially pure human voltage-gated calcium channel $\alpha 2\delta$-2 isoforms designated $\alpha 2\delta 2$-a and $\alpha 2\delta 2$-b subunit polypeptides, preferably comprising the amino acid sequence of SEQ ID NO:2 and 4 respectively, each of which, in turn, is encoded by the novel isolated nucleic acid molecules described above – SEQ ID NO:1 and SEQ ID NO:3. "$\alpha 2\delta 2$-a or $\alpha 2\delta 2$-b splice variant(s)" collectively referred to as the "$\alpha 2\delta$-2 isoforms" or "splice variants" – is an amino acid sequence encoded by the $\alpha 2\delta$-2 variant nucleic acid sequences, each of which is a translation product of naturally occurring mRNA sequence obtained as a result of alternative splicing. The amino acid sequence may be a peptide, a protein, as well as peptides or proteins having chemically modified amino acids such as a glycopeptide or glycoprotein. The term also includes homologues of said sequences in which one or more amino acids has been added, deleted, substituted or chemically modified as well as fragments of this sequence having at least 85% sequence similarity with the splice variants disclosed herein, with the proviso that said fragments are functionally equivalent or biologically equivalent to the novel isoform of the invention from which the fragment is

derived from. "Polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residues is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

Significantly, the novel proteins of the invention include "altered" or "variant" proteins which may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent or biologically equivalent isoform of $\alpha 2\delta$-2 subunit, e.g., functionally equivalent to the protein of SEQ ID NO:2 or fragments thereof. Deliberate amino acid substitutions or conservative amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of a human voltage-gated calcium channel subunit is retained. Altered isoforms of the invention can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, e.g. Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, or Current Protocols in Molecular Biology, F. M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Exemplary functionally equivalent variants of the human voltage-gated calcium channel $\alpha 2\delta 2$-a subunit polypeptides include conservative amino acid substitutions of SEQ ID NO:2. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D. Thus, for example, example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

Consequently, the novel $\alpha 2\delta$-2 subunit polypeptide isoforms include any human voltage-gated calcium channel clone having the amino acid sequence as set forth on in SEQ ID NO:2 or SEQ ID NO:4 or a biologically active fragment thereof or one having an amino acid sequence that is substantially similar to one of SEQ ID NO:2 or 4. Importantly, the foregoing discussion details the $\alpha 2\delta 2$-a subunit (SEQ ID NO:1 and 2), it is understood that the same discussion applies equally to the $\alpha 2\delta 2$-b subunit (SEQ ID NO:3 and 4).

As used herein, an "altered" human voltage-gated calcium channel $\alpha 2\delta$-2 isoform is a polypeptide which contains one or more modifications to the primary amino acid sequence of a human voltage-gated calcium channel $\alpha 2\delta$-2 isoform of the invention, e.g., SEQ ID NO:2.

In this context, "altered" isoforms of the invention refer to those polypeptides that are biologically equivalent (active) to the polypeptides of SEQ ID NO:2, for example, meaning that the altered protein retains some functional characteristics of the full-length polypeptide, e.g., antigenicity, or structural domain *vis-a-vis* ion channel domain, its ability to regulate voltage-gated calcium influx etc. Whether a particular variant protein is biologically equivalent to a target protein can be assessed based upon some physiologically measurable effect on target cells, molecules or tissues. Biological activity can also be demonstrated in a number of assays, e.g., patch clamp etc well known to one skilled in the art. For example, a biologically equivalent variant or altered $\alpha 2\delta 2$-a subunit polypeptide retains the ability to bind ligand and/or transduce a voltage-gated calcium current as does the protein of SEQ ID NO:2. Other functionally equivalent variants will be known to one of ordinary skill in the art, as will methods for preparing such variants.

The aforementioned modifications which result in an altered human voltage-gated calcium channel $\alpha 2\delta$-2 isoform can be made to the novel splice variant proteins of the invention for a variety of reasons, including 1) to reduce or eliminate an activity of a human voltage-gated calcium channel subunit, e.g., $\alpha 2\delta 2$-a subunit polypeptide, such as voltage-gated calcium influx; 2) to enhance a property of a human voltage-gated calcium channel $\alpha 2\delta 2$-a subunit polypeptide, such as protein stability in an expression system or the stability of protein-protein binding; 3) to provide a novel activity or property to a human voltage-gated calcium channel $\alpha 2\delta 2$-a subunit polypeptide, such as addition of an antigenic epitope/ antigenic peptide or addition of a detectable moiety; or 4) to establish that an amino acid substitution does or does not affect voltage-gated calcium influx. Such modifications are typically made to the nucleic acid which encodes the target human voltage-gated calcium channel $\alpha 2\delta$-2 subunit polypeptide, e.g., SEQ ID NO:2 or 4 and can include deletions, point mutations, truncations, amino acid substitutions and additions of amino acids or non-amino acid moieties. In the alternative, the proposed modifications can be made directly to the target polypeptide, such as by cleavage, addition of a linker molecule, addition of a detectable moiety, such as biotin, addition of a fatty acid, and the like. Modifications further embrace fusion proteins comprising all or part of the amino acid of SEQ ID NO:2 for example.

One of skill in the art is deemed to be familiar with methods for predicting the effect on protein conformation of a change in protein sequence, and can thus "design" an "altered" isoform embraced by the invention according to known methods. See, e.g., Dahiyat and Mayo, Science 278:82-87, 1997, for one such exemplary method whereby proteins can be designed *de novo*. The method can be applied to a known protein to vary only a portion of the polypeptide sequence. By applying the computational methods of Dahiyat and Mayo, specific

variants of a cancer associated antigen polypeptide have been proposed and tested to determine whether the variant retains a desired conformation.

Preferred altered is a modified or altered form of one of the α2δ-2 isoforms of the invention, e.g., α2δ2-a subunit polypeptide which is modified specifically to alter a feature of the polypeptide unrelated to its physiological activity. For example, cysteine residues can be substituted or deleted to prevent unwanted disulfide linkages. Similarly, certain amino acids can be changed to enhance expression of the protein by eliminating proteolysis by proteases in an expression system (e.g., dibasic amino acid residues in yeast expression systems in which KEX2 protease activity is present).

Mutations of a nucleic acid which encode an altered isoform of the invention preferably preserve the amino acid reading frame of the coding sequence, and preferably do not create regions in the nucleic acid which are likely to hybridize to form secondary structures, such as hairpins or loops, which can be deleterious to expression of the variant polypeptide.

Mutations can be made by specifically selecting an amino acid substitution, or by random mutagenesis of a selected site in a nucleic acid which encodes the target polypeptide. Altered polypeptides are then expressed and tested for one or more activities to determine which mutation provides the target protein with a desired property. Additional mutations can be made to the altered isoform or the corresponding reference splice variant of the invention e.g., altered α2δ2-a subunit or to the corresponding protein of SEQ ID NO:2 , which are silent as to the amino acid sequence of the polypeptide, but which provide preferred codons for translation in a particular host. The preferred codons for translation of a nucleic acid in, e.g., E. coli, are known to those of ordinary skill in the art. Still other mutations can be made to the noncoding sequences of a human voltage-gated calcium channel α2δ2-a subunit gene or cDNA clone to enhance expression of the polypeptide.

The activity of an altered isoform of the invention can be tested by cloning the gene encoding the altered human voltage-gated calcium channel e.g., α2δ2-a isoform subunit polypeptide into a bacterial or mammalian expression vector, introducing the vector into an appropriate host cell, expressing the desired target polypeptide, and testing it for a functional capability characteristic of the corresponding α2δ2 isoform of the invention, e.g., the α2δ2-a subunit polypeptide. For example, the altered α2δ2-a subunit polypeptide isoform can be tested for ability to provide voltage regulated calcium influx. Preparation of other altered polypeptides may favor testing of other activities, as will be known to one of ordinary skill in the art. See also the method detailed in Lin et al., Neuron 18:153-166, 1997, and U.S. Pat. No. 5,429,921, to Harpold et al., entitled "Assays for agonists and antagonists of recombinant human calcium channels". Altered α2δ-2 subunit polypeptides, derived from either one of the novel isoforms

disclosed herein (SEQ ID NO:2 or 4) will find use, *inter alia*, in assays for identifying compounds that bind and/or regulate the calcium influx function of the corresponding $\alpha 2\delta$-2 isoform of the invention, e.g., SEQ ID NO:2. Such variants will also be useful for determining the portions of the corresponding channel $\alpha 2\delta 2$ isoform, e.g., SEQ ID NO:2 that is required for calcium influx activity. Non-functional variants, on the other hand, will find use, for example, as antagonists of calcium channel function and as negative controls in experiments designed to test subunit activity etc.

In a preferred embodiment, the biologically active variants of the invention include proteins having an amino acid sequence sharing at least about 70% amino acid sequence identity with the splice variants of the invention, preferably at least about 75%, more preferably at least about 80%, still more preferably at least about 85%, even more preferably at least about 90%, and most preferably at least about 95%. Amino acid substitutions are preferably substitutions of single amino-acid residues.

"Identity" or "homology" with respect to the variant polypeptides, detailed above, is defined herein as the percentage of amino acid residues in the candidate sequence (variant) that are identical with the residues in the reference protein, e.g., SEQ ID NO:2, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. No N- nor C-terminal extensions, deletions nor insertions shall be construed as reducing identity or homology.

Parameters for polypeptide sequence comparison include the following:

1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison Wis. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50,60, 70, 80, 85, 90, or 95% identity to a reference polypeptide, wherein the variant polypeptide sequence may be identical to the reference sequence of SEQ ID NO:2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein the alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or

insertion, and wherein the alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein the number of amino acid alterations is determined by multiplying the total number of amino acids in the reference sequence, e.g., SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from the total number of amino acids in SEQ ID NO:2, or:

Na = Xa - (Xa Y), wherein Na is the number of amino acid alterations, Xa is the total number of amino acids in SEQ ID NO:2, Y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and is the symbol for the multiplication operator, and wherein any non-integer product of Xa and Y is rounded down to the nearest integer prior to subtracting it from Xa.

A variety of methodologies well-known to the skilled practitioner can be utilized to obtain isolated human voltage-gated calcium channel $\alpha 2\delta$-2 subunit isoforms detailed herein, e.g., $\alpha 2\delta 2$-a subunit. The polypeptide may be purified from cells which naturally produce the polypeptide by chromatographic means or immunological recognition. Alternatively, an expression vector may be introduced into cells to cause production of the polypeptide. In another method, mRNA transcripts may be microinjected or otherwise introduced into cells to cause production of the encoded polypeptide. Translation of mRNA in cell-free extracts such as the reticulocyte lysate system also may be used to produce polypeptide. Those skilled in the art also can readily follow known methods for isolating one or both human voltage-gated calcium channel $\alpha 2\delta$-2 isoforms of the invention. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography and immune-affinity chromatography.

A fragment or a biologically active or equivalent fragment of the splice variant proteins of the invention are also embraced by the invention. Such a "fragment" is meant to refer to a protein which contains a portion of the complete amino acid sequence of the reference protein, e.g., SEQ ID NO:2. Such molecules are expected to have, *inter alia*, similar biological functions/properties equivalent to the polypeptide from which the fragment is derived.

Preferred fragments are those which retain a distinct functional capability of the corresponding reference splice variant of the invention from which the fragment is derived. Exemplary functions include voltage regulated calcium influx. Other functional capabilities which can be retained in a fragment of either of the splice variants of the invention include the ability to interact with antibodies as well as with other polypeptides (such as other subunits of the human voltage-gated calcium channel) and binding partners. Those skilled in the art are well

versed in methods for selecting fragments which retain a functional capability of the reference splice variant. Functional capability of the target fragment can be confirmed by synthesizing the fragment and testing of the capability according to standard methods. For example, when testing the fragment for its ability to voltage regulate calcium influx, a skilled artisan may transform and express the target fragment in a cell in which calcium influx can be measured. Such methods, are standard in the art.

The splice variant polypeptides of the invention polypeptide, functional equivalents thereof, and biologically active fragments, can also be produced by chemical synthesis. For example, synthetic polypeptides can be produced using Applied Biosystems, Inc. Model 430A or 431A automatic peptide synthesizer (Foster City, Calif.) employing the chemistry provided by the manufacturer.

The present invention also provides compositions containing an acceptable carrier and any of an isolated, purified splice variants of the invention, an active fragment thereof, or a purified, mature protein and active fragments thereof, alone or in combination with each other. These polypeptides or proteins can be recombinantly derived, chemically synthesized or purified from native sources.

Antibodies and Uses Therefor - The splice variant polypeptides of the invention or a immunologically active fragment/antigenic peptides thereof may be used as immunogens to generate an antibody or a functionally active antibody fragment specific for one of the splice variant polypeptides disclosed herein. (See, for example, Antibodies: A Laboratory Manual ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). The subject antibodies, in turn, can be used for producing hybridoma(s), and identifying pharmaceutical compositions, and for studying DNA/protein interaction.

The antigenic peptide fragment of the herein disclosed human voltage-gated calcium channel $\alpha 2\delta$-2 isoforms, e.g., a $\alpha 2\delta 2$-a subunit comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 and encompasses an epitope of the subject $\alpha 2\delta 2$-a such that an antibody raised against the peptide forms a specific immune complex with the subject $\alpha 2\delta 2$-a from which it was derived. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues. Methods of determining the antigenic determinant of an antibody or peptide fragment thereof are known and may be employed to obtain synthetic antigenic peptides useful in accordance with the present invention. See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983).

Consequently, an embodiment of the invention contemplates an intact, fully human anti-human voltage-gated calcium channel $\alpha_2\delta 2$–a subunit monoclonal antibody in an isolated form or in a pharmaceutical preparation. Methods for developing a monoclonal antibody that interacts with and inhibits the activity of the subject human voltage-gated calcium channel $\alpha_2\delta 2$–a subunit are well known. A representative method of developing such an antibody is provided herein, it being understood that this method is provided for illustrative purposes only.

The term "antibody" is used in the broadest sense and specifically covers single monoclonal antibodies (including agonist and antagonist antibodies) and antibody compositions with polyepitopic specificity. In general, the term refers to immunoglobulin molecules and antigenically or immunologically active fragments of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as for example, the $\alpha_2\delta 2$-a subunit polypeptide. It is understood that the term "antibody" includes not only intact immunoglobulin molecules but also the well-known active fragments $F(ab')_2$, and Fab. $F(ab')_2$, and Fab fragments which lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., J. Nucl. Med. 24:316-325 (1983)).

Methods of making antibodies are well known. For example, polyclonal anti-$\alpha_2\delta 2$-a subunit antibodies can be prepared by immunizing a suitable subject with a $\alpha_2\delta 2$-a subunit immunogen. Various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with $\alpha_2\delta 2$-a subunit or an immunologically active fragment(s) thereof for producing the antibodies. Alternatively, short stretches of $\alpha_2\delta 2$-a subunit amino acids may be fused with those of another protein to form chimeric entities, and antibodies to the chimeric entity may then produced. At an appropriate time after immunization, e.g., when the anti-$\alpha_2\delta 2$-a subunit antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) Nature 256:495-497) (see also, Brown et al. (1981) J. Immunol. 127:539-46; Brown et al. (1980) J. Biol. Chem. 255:4980-83; Yeh et al. (1976) Proc. Natl. Acad. Sci. USA 76:2927-31; and Yeh et al. (1982) Int. J. Cancer 29:269-75), the more recent human B cell hybridoma technique (Kozbor et al. (1983) Immunol Today 4:72), the EBV-hybridoma technique (Cole et al. (1985), Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. A common technique entails fusing an immortal cell line (typically a myeloma) to lymphocytes (typically splenocytes) from a mammal immunized with a $\alpha_2\delta 2$-a subunit immunogen as described above,

followed by screening the culture supernatants of the resulting hybridoma cells to identify a hybridoma producing a monoclonal antibody that binds $\alpha 2\delta 2$-a subunit.

Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynabacterium parvum are especially preferable.

An appropriate immunogenic preparation can contain, for example, recombinantly expressed $\alpha 2\delta 2$-a subunit protein or a chemically synthesized $\alpha 2\delta 2$-a subunit protein. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic $\alpha 2\delta 2$-a subunit preparation induces a polyclonal anti-$\alpha 2\delta 2$-a subunit antibody response.

The prior art is replete with methods of preparing human monoclonal antibodies. Representative methods are disclosed in U.S. Pat. No. 5,567,610, issued to Borrebaeck et al., U.S. Pat. No. 5,565,354, issued to Ostberg, U.S. Pat. No. 5,571,893, issued to Baker et al, Kozber, J. Immunol. 133: 3001 (1984), Brodeur, et al., Monoclonal Antibody Production Techniques and Applications, p. 51-63 (Marcel Dekker, Inc, new York, 1987), and Boerner et al., J. Immunol., 147: 86-95 (1991). As well, such antibodies may also be prepared by immunizing transgenic animals that are capable of producing human antibodies (e.g., Jakobovits et al., Proc. Nat'l. Acad. Sci. USA, 90: 2551 (1993), Jakobovits et al., Nature, 362: 255-258 (1993), Bruggermann et al., Year in Immuno., 7:33 (1993) and U.S. Pat. No. 5,569,825 issued to Lonberg).

One well versed in the art of immunology is well aware that only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W. R. (1986) The Experimental Foundations of Modern Immunology Wiley & Sons, Inc., New York; Roitt, I. (1991) Essential Immunology, 7th Ed., Blackwell Scientific Publications, Oxford). For example, it is well accepted that the pFc' and Fc regions of an antibody are not involved in antigen binding but instead play a significant role in the complement cascade. Significantly, an antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an $F(ab')_2$ fragment, retains both of the antigen binding sites of an intact antibody. Likewise, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Fab fragments are composed of a covalently bound antibody light

chain and a portion of the antibody heavy chain denoted Fd, which is the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

As well, it is well accepted that within the antigen-binding portion of an antibody, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three (3) complementarity determining regions (CDR1 through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, are largely responsible for antibody specificity.

Conventional immunoassays may be employed to identify antibodies having the desired specificity. Such protocols include but are not limited to those described in U.S. Patent Nos. 4,642,285; 4,376,110; 4,016,043; 3,879,262; 3,852,157; 3,850,752; 3,839,153; 3,791,932; and Harlow and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, N.Y. (1988), each incorporated by reference herein. Likewise, the sequences of the antigen-binding sites of an anti-$\alpha_2\delta 2$-a subunit monoclonal antibodies once identified by any one or more of the above referenced assays can be determined using amino acid sequencing techniques that are routine in the art. The same can be done with the relevant FR and CDR regions.

A skilled artisan well versed in immunology is also aware that one may increase the specificity/affinity/avidity of an antibody for its specific antigen by "humanizing" the subject antibody. Techniques to humanize antibodies are also well known to one skilled in the art of immunology. The technique is particularly useful when non-human animal (e.g., murine) antibodies which inhibit a target human voltage-gated calcium channel subunit, e.g., the $\alpha_2\delta 2$-a subunit of the invention are identified. It is also known that non-CDR regions of a mammalian antibody may be replaced with corresponding regions of non-specific or hetero-specific antibodies while retaining the epitope specificity of the original antibody is well known. This method proposes covalently joining non-human CDRs (e.g., murine) to human FR and/or Fc/pFc' regions to produce a functional antibody. The resulting "humanized" antibodies can be used in the treatment of a human subject in the methods according to the invention. The proposed chimeric and humanized monoclonal antibodies can also be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson et al. International Application No. PCT/US86/02269; Akira, et al. European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al. European Patent

Application 173,494; Neuberger et al. PCT International Publication No. WO 86/01533; Cabilly et al. U.S. Pat. No. 4,816,567; Cabilly et al. European Patent Application 125,023; Better et al. (1988) Science 240:1041-1043; Liu et al. (1987) Proc. Natl. Acad. Sci. USA 84:3439-3443; Liu et al. (1987) J. Immunol. 139:3521-3526; Sun et al. (1987) Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura et al. (1987) Canc. Res. 47:999-1005; Wood et al. (1985) Nature 314:446-449; and Shaw et al. (1988) J. Natl. Cancer Inst. 80:1553-1559); Morrison, S. L. (1985) Science 229:1202-1207; Oi et al. (1986) BioTechniques 4:214; Winter U.S. Pat. No. 5,225,539; Jones et al. (1986) Nature 321:552-525; Verhoeyan et al. (1988) Science 239:1534; and Beidler et al. (1988) J. Immunol. 141:4053-4060. An example of a method for humanizing a murine antibody is provided in U.S. Patent Nos. 5,530,101 and 5,585,089 to Queen et al. See also PCT International Publication No. WO 92/04381 which teaches the production and use of humanized murine RSV antibodies in which at least a portion of the murine FR regions have been replaced by FR regions of human origin. Such antibodies, including fragments of intact antibodies with antigen-binding ability, are often referred to as "chimeric" antibodies.

Reportedly, the above described humanized antibodies are capable of provoking an immune response against the non-human sequences (HAMA response). Consequently, such antibodies are not preferred for use in immuno-compromised humans and are particularly not preferred for extended use. However, the HAMA response may be lessened or abolished by techniques well known to one skilled in the art of immunology. Indeed, the prior art is replete with means of lessening or abolishing the HAMA response. Preferably, the chimeric antibodies of the invention are fully human monoclonal antibodies, where some or all of the FR regions of the antibody are replaced by other homologous human FR regions. A particular utility of the fully human chimeric antibodies is that they will not evoke an immune response against the antibody itself.

In accordance with the above, an embodiment of the invention provides F(ab')2, and Fab fragments of an anti-$\alpha 2\delta 2$-a subunit monoclonal antibody; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions of the anti-$\alpha 2\delta 2$-a subunit antibody have been replaced by homologous human or non-human sequences; chimeric F(ab')2 fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions of an anti-$\alpha 2\delta 2$–a subunit antibody have been replaced by homologous human or non-human sequences; and chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences. It is understood that a skilled artisan may alter an anti-$\alpha 2\delta 2$-a subunit antibody by CDR grafting or produce chimeric antibodies or antibody fragments containing, all or part thereof, of the disclosed heavy and light chain V-region CDR amino acid sequences

(Jones et al., Nature 321:522, 1986; Verhoeyen et al., Science 39:1534, 1988 and Tempest et al., Bio/Technology 9:266, 1991), without destroying the specificity of the antibodies for either one of the human voltage-gated calcium channel isoforms of the invention. Such CDR grafted or chimeric antibodies or antibody fragments can be very effective in inhibiting human voltage-gated calcium channel $\alpha 2\delta 2$-a subunit activity in animals (e.g. primates) and humans.

Fab fragments, including chimeric Fab fragments, are preferable in those methods where the antibodies of the invention are administered directly to a local tissue environment. For example, the Fab fragments are preferred when the antibody of the invention is administered directly to the brain. Fabs offer several advantages over $F(ab')_2$ and whole immunoglobulin molecules for this therapeutic modality. That Fab molecules of the invention are preferable for the above purposes becomes evident when one considers that since Fabs have only one binding site for their cognate antigen, the formation of immune complexes is precluded in contrast to the generation of such complexes when using bivalent $F(ab')_2$ s and whole immunoglobulin molecules upon their encounter with the target antigen. Of equal import is the observation that immune complex deposition in tissues can produce adverse inflammatory reactions. As well, the absence of a Fc region in Fabs prevents an adverse inflammatory reactions that generally attends antibodies with an Fc, whose activation, in turn, may activate the complement cascade. Also, it is believed that tissue penetration and bioavailability of the small Fab molecule is likely to be much better than that of the larger whole antibody. More, it is cheaper and easier to produce Fabs, e.g., in bacteria, such as E. coli, whereas whole immunoglobulin antibody molecules require mammalian cells for their production in useful amounts. Indeed, production of Fabs in *E. coli* makes it possible to produce these antibody fragments in large fermenters which are less expensive than cell culture-derived products. For inoculation or prophylactic use, the antibodies of the present invention should preferably be intact antibody molecules including the Fc region. Such intact antibodies generally exhibit longer half-lives than smaller fragment antibodies (e.g. Fab) and are more suitable for intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous, or transdermal administration.

In addition to the above, smaller antibody fragments and small binding peptides having binding specificity for a human voltage-gated calcium channel $\alpha 2\delta$-2 isoform of the invention will find use as inhibitors of human voltage-gated calcium channel $\alpha 2\delta$-2 activity and are thus also embraced by the invention. For example, single-chain antibodies can be constructed in accordance with the methods described in U.S. Pat. No. 4,946,778 to Ladner et al. Such single-chain antibodies include the variable regions of the light and heavy chains joined by a flexible linker moiety. Methods for obtaining a single domain antibody ("Fd") which comprises an isolated VH single domain are detailed in Ward et al., Nature 341:644-646 (1989)).

Methods for assessing the affinity of antibodies for a $\alpha 2\delta$-2 subunit protein isoform, e.g., $\alpha 2\delta 2$-a are also well known. For example, Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies of the invention. Affinity is expressed as an association constant, K_a, which is defined as the molar concentration of $\alpha 2\delta$-2 isoform specific antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple $\alpha 2\delta$-2 isoform subunit epitopes, represents the average affinity, or avidity, of the antibodies for the respective $\alpha 2\delta$-2 subunit. The K_a determined for a preparation of monoclonal antibodies, which are monospecific for a particular $\alpha 2\delta$-2 isoform epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} l/mole are preferred for use in immunoassays in which the $\alpha 2\delta$-2 isoform -antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 l/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of the respective $\alpha 2\delta$-2 isoform, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington D.C.; Liddell, J. E. and Cryer, A. (1991) A Practical Guide to Monoclonal Antibodies, Johne Wiley & Sons, New York N.Y.).

Immunological procedures useful for *in vitro* detection of a $\alpha 2\delta$–2 isoform, e.g., $\alpha 2\delta 2$-a (SEQ ID NO:2) in a sample are well known. Indeed, numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are known to one skilled in the art. Such immunoassays include, for example, ELISA, Pandex microfluorimetric assay, agglutination assays, flow cytometry, serum diagnostic assays and immunohistochemical staining procedures, which are well known in the art. Typically, the immunoassay involves the measurement of complex formation between the target $\alpha 2\delta$-2 isoform subunit and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering $\alpha 2\delta$-2 isoform subunit epitopes is preferred, but a competitive binding assay may also be employed. The antibodies may be made detectable by various means well known in the art. For example, a detectable marker can be directly or indirectly attached to the antibody. Useful markers include, for example, radionuclides, enzymes, fluorogens, chromogens and chemiluminescent labels.

III. Proposed uses for the human voltage-gated calcium channel $\alpha 2\delta$-2 subunit isoform polypeptide and polynucleotide encoding same.

The invention as described herein is useful for a variety of therapeutic and non-therapeutic purposes. For example, the invention permits isolation of an isoform of a human voltage-gated calcium channel $\alpha_2\delta$-2 subunit polypeptide molecule, e.g., $\alpha_2\delta$2-a subunit polypeptide containing the amino acid sequence of SEQ ID NO:2, by for example expression of a recombinant nucleic acid to produce large quantities of the desired polypeptide which may be isolated using standard protocols. Likewise, polynucleotides comprising a nucleotide sequence depicted in SEQ ID NO:1 or a sequence substantially similar to SEQ ID NO:1 may also be isolated using the polynucleotides disclosed herein.

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding, for example, the $\alpha_2\delta$2-a subunit polypeptide (SEQ ID NO:2) include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding the $\alpha_2\delta$2-a subunit polypeptide (SEQ ID NO:2), or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Pharmacia & Upjohn (Kalamazoo, Mich.). Promega (Madison, Wis.), and U.S. Biochemical Corp. (Cleveland, Ohio). Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

As such, portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) detect $\alpha_2\delta$2-a subunit encoding nucleotide sequences in a sample; and (ii) map their respective genes on a chromosome, and, thus, locate gene regions associated with genetic disease. Gene products can likewise be used as herein described.

The binding or interaction of a candidate compound with a receptor or fragments thereof e.g., $\alpha_2\delta$2-a can also be measured directly by using radioactively labeled compound of interest or by the second messenger effect resulting from the interaction or binding of the candidate compound. (See, e.g., Lazereno and Birdsall (1993) Br. J. Pharmacol. 109:1120-1127.) Modulation in receptor signaling can be measured using a detectable assay, e.g., the FLIPR assay. (See, e.g., Coward, P. (1999) Anal. Biochem. 270:242-248; Sittampalam, supra; and Gonzalez and Negulescu, supra.) Activation of certain receptors, in particular, GPCRs, can

be measured by an ^{35}S-GTPγS binding assay. (See, e.g., Lazareno (1999) Methods Mol. Biol. 106:231-245.) Alternatively, the candidate compounds can be subjected to competition screening assays, in which a known ligand, preferably labeled with an analytically detectable reagent, most preferably radioactivity, is introduced with the drug to be tested and the capacity of the compound to inhibit or enhance the binding of the labeled ligand is measured. Compounds are screened for their increased affinity and selectivity for the specific receptor or fragments thereof.

Transcription based assays that identify signals that modulate the activity of cell surface proteins, e.g., receptors, ion channels, etc., may be used to screen candidate compounds for their ability to stimulate reporter gene product expression and their potential to stimulate the expression of a human voltage-gated -calcium channel α2δ-2 isoform, e.g., α2δ2-a. Transcription-based assays are well known in the art. (See, e.g., Zlokarnik, et al. (1998) Science 279:84-88; Siverman, supra; and Gonzalez and Negulescu, (1998) Curr. Opin. Biotechnol. 9:624-631.) These transcription based assays asses the intracellular transduction of an extracellular signal using recombinant cells that are modified by introduction of a reporter gene under the control of a regulatable promoter.

Determining the ability of an α2δ-2 isoform of the invention, e.g. the α2δ2-a subunit polypeptide (SEQ ID NO:2) to bind to or interact with a α2δ2-a target molecule (binding partner/binding peptide) can be accomplished, for example, by coupling the α2δ2-a subunit with a radioisotope or enzymatic label such that binding of the α2δ2-a subunit to a α2δ2-a target molecule can be determined by detecting the labeled α2δ2-a subunit in a complex. See, e.g., Wainscott et al. (1993) Mol. Pharmacol. 43:419-426; and Loric, et al. (1992) FEBS Lett. 312:203-207.) For example, the α2δ2-a subunit, can be labeled with 125I, 35S, 14C, or 3H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, α2δ2-a subunit molecules can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

A two-hybrid system-based approach can also be employed for compound screening, small molecule identification, and drug discovery. The underlying premise of the two-hybrid system, originally described in yeast by Fields and Song (1989) Nature 340:245-246, provides a connection between a productive protein-protein or protein-compound interaction pair of interest and a measurable phenotypic change in yeast. A reporter cassette containing an up-stream activation sequence which is recognized by a DNA binding domain, is operationally linked to a reporter gene, which when expressed under the correct conditions will generate a

phenotypic change. The original two-hybrid system has recently been modified for applicability in high-throughput compound screening. (See, e.g., Ho et al. (1996) Nature 382:822-826; Licitra and Liu (1996) Proc. Natl. Acad. Sci. USA 93:12817-12821; and Young et al. (1998) Nature Biotech. 16:946-950.)

The invention also features binding peptides (agents) that bind to the $\alpha 2\delta$-2 isoforms disclosed herein. These peptides may be derived from a variety of sources. For example, binding peptides may easily be synthesized or produced by recombinant means by those of skill in the art. Using routine procedures known to those of ordinary skill in the art, one can determine whether a peptide which binds to a human voltage-gated calcium channel $\alpha 2\delta$-2 subunit isoform of the invention is useful by determining whether the peptide inhibits the activity of the respective $\alpha 2\delta$-2 isoform in a voltage-gated calcium influx assay, as discussed above.

The binding peptides according to the invention may also be an antibody or a functionally active antibody fragment. Antibodies are well known to those of ordinary skill in the science of immunology. Such fragments are also well known in the art and are regularly employed both *in vitro* and *in vivo*. In particular, as used herein, the term "antibody" means not only intact immunoglobulin molecules but also the well-known active fragments F(ab').sub.2, and Fab. F(ab').sub.2, and Fab fragments which lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., J. Nucl. Med. 24:316-325 (1983)).

The invention also embraces agents which bind selectively to the one of the herein disclosed human voltage-gated calcium channel $\alpha 2\delta$-2 subunit polypeptide isoform , e.g., SEQ ID NO:2 or SEQ ID NO:4 and agents which bind preferentially to either one or both of the isoforms disclosed herein. Agents which bind to the isoforms of SEQ ID NO:2 or 4 and fragments of the aforementioned polypeptides and nucleic acid molecules are also included within the scope of the invention.

Selective binding means that the agent binds to, for example, a the human voltage-gated calcium channel $\alpha 2\delta 2$-a subunit but not to non-$\alpha 2\delta 2$-a or $\alpha 2\delta 2$-b (i.e., those subunits which do not have or encode SEQ ID NO:2 or 4). Preferential binding means that the agent binds more to the human voltage-gated calcium channel $\alpha 2\delta 2$-a (SEQ ID NO:2) or $\alpha 2\delta 2$-b subunit (SEQ ID NO:4) than to human voltage-gated calcium channel non-$\alpha 2\delta$-2 subunit, e.g., the agent binds with greater affinity or avidity to the human voltage-gated calcium channel $\alpha 2\delta 2$-a subunit isoforms disclosed herein. The agents can inhibit or increase activity of the respective isoform polypeptide - (antagonists and agonists, respectively).

For example, in order to determine whether an $\alpha 2\delta$-2 isoform specific binding peptide binds to, e.g., the $\alpha 2\delta 2$-a subunit any known binding assay may be employed. For example, the peptide may be immobilized on a surface and then contacted with a labeled $\alpha 2\delta 2$-a subunit. The amount of $\alpha 2\delta 2$-a subunit which interacts with the binding peptide or the amount which does not bind to the $\alpha 2\delta 2$-a subunit binding peptide may then be quantitated to determine whether the binding peptide binds to the subject $\alpha 2\delta 2$–a subunit. Further, the binding of the $\alpha 2\delta 2$-a subunit and a non-$\alpha 2\delta 2$-a subunit can also be compared to determine if the binding peptide binds selectively or preferentially.

Consequently, in screening for modulators of the $\alpha 2\delta 2$-a subunit (SEQ ID NO:2) it is preferred that compounds (e.g. synthetic combinatorial libraries, natural products, peptide libraries, etc.) are tested for modulation of $\alpha 2\delta 2$-a subunit activity at a variety of voltages which cause partial or complete membrane depolarization, or hyperpolarization. These assays are conducted according to standard procedures of testing calcium channel function (e.g. patch clamping, fluorescent Ca^{2+} influx assays) which require no more than routine experimentation. Using such methods, modulators of $\alpha 2\delta 2$-a subunit activity which are active at particular voltages (e.g. complete membrane depolarization) can be identified. Such compounds are useful for selectively modulating calcium channel activity in conditions which may display voltage dependence.

Other assays for identifying compounds that modulate ion channel activity are practiced by measuring the ion channel activity when a cell expressing the ion channel of interest, or fragments thereof, is exposed to a solution containing the test compound and a ion channel selective ion and comparing the measured ion channel activity to the native ion channel activity of the same cell or a substantially identical control cell in a solution not containing the test compound. Methods for practicing such assays are known to those of skill in the art. (See, e.g., Mishina et al. (1985) Nature 313:364-369; and Noda, et al. Nature 322:836-828.)

In addition, an aspect of the invention is drawn to the use of the novel nucleic acid molecules to map the location of the gene on a chromosome. This process is called chromosome mapping.

Probes derived from the novel polynucleotides sequences disclosed herein can be used herein to identify genomic or cDNA library clones possessing an identical or substantially similar nucleic acid sequence. A suitable oligonucleotide or set of oligonucleotides, which is capable of hybridizing under stringent hybridization conditions to the desired sequence, a variant or fragment thereof, or an anti-sense complement of such an oligonucleotide or set of oligonucleotides, can be synthesized by means well known in the art (see, for example, Synthesis and Application of DNA and RNA, S. A. Narang, ed., 1987, Academic Press, San

Diego, Calif.) and employed as a probe to identify and isolate the desired sequence, variant or fragment thereof by techniques known in the art. Techniques of nucleic acid hybridization and clone identification are disclosed by Sambrook, et al., Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y. (1989), and by Hames, B. D., et al., in Nucleic Acid Hybridization, A Practical Approach, IRL Press, Washington, D.C. (1985). Means for producing specific hybridization probes for detecting identical or related sequences are well known. mRNA probes may also be used, whose method of preparation is well known to a person well versed in the art of cell biology. Vectors for the production of mRNA probes are commercially available, and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides.

To facilitate the detection of a desired nucleic acid sequence, or variant or fragment thereof, whether for cloning purposes or for the mere detection of the presence of the sequence, the above-described probes may be labeled with a detectable group. Such a detectable group may be any material having a detectable physical or chemical property. Such materials have been well-developed in the field of nucleic acid hybridization and, in general, most any label useful in such methods can be applied to the present invention.

In one aspect, the invention proposes the use of the hybridization probes for mapping the naturally occurring genomic sequence. Fragments of the $\alpha 2\delta$-2 isoform encoding sequences (SEQ ID NO:1 or 3) may be used to map these genes to the appropriate mouse and human chromosomes. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome or to artificial chromosome constructions, such as human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Price, C. M. (1993) Blood Rev. 7:127-134, and Trask, B. J. (1991) Trends Genet. 7:149-154.)

Briefly, the sequence(s) is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Upon mapping a sequence to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. et al. Nature, 325:783-787 (1987). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a

mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

For example, $\alpha 2\delta 2$-a subunit encoding genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the herein disclosed $\alpha 2\delta 2$-a nucleotide sequence. Computer analysis of the $\alpha 2\delta 2$-a subunit sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. Thereafter, these primers can be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the $\alpha 2\delta 2$-a subunit sequences will yield an amplified fragment.

Somatic cell hybrids containing only fragments of human chromosomes can be produced by using human chromosomes with translocations and deletions. PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using $\alpha 2\delta 2$-a subunit nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel will contain either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. et al. (1983) Science 220:919-924).

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Verma et al. (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, N.Y.) Examples of genetic map data can be found in various scientific journals or at Online Mendelian Inheritance in Man (OMIM). Correlation between the location of the gene encoding $\alpha 2\delta 2$-a on a physical chromosomal map and a specific disease, or predisposition to a specific disease, may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier, or affected individuals. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle.

Consequently, fluorescence in situ hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step.

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

More, differences in the DNA sequences between individuals affected and unaffected with a disease associated with a human voltage-gated calcium channel $\alpha_2\delta$-2 subunit or an isoform thereof such as the ones disclosed herein can also be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then it is save to assume that the mutation is likely the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Other mapping strategies include in situ hybridization (described in Fan, Y. et al. Proc. Natl. Acad. Sci. USA, 87:6223-27(1990)), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries. In situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region (see, e.g., Gatti, R. A. et al. (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

To detect nucleic acid encoding a polypeptide of the invention or one having the sequence substantially as set forth in SEQ ID NO:1, standard hybridization and/or PCR

techniques may be employed using a nucleic acid probe or a PCR primer. Suitable probes and primers may be designed by those of ordinary skill in the art based on the α2δ2 isoform subunit cDNA sequences provided herein.

Consequently, determining the level of , e.g., α2δ2-a subunit (SEQ ID NO:1) expression may be accomplished by Northern blot analysis. Polyadenylated [poly(A)+] mRNA is isolated from a test sample. The mRNA is fractionated by electrophoresis and transferred to a membrane. The membrane is probed with labeled α2δ2-a subunit cDNA. In another embodiment, α2δ2-a subunit expression is measured by quantitative PCR applied to expressed mRNA. In yet another embodiment, the test reagent is incubated with a cell transfected with an α2δ2-a subunit polynucleotide expression vector, and the effect of the test reagent on α2δ2-a subunit transcription is measured by Northern blot analysis, as described above.

In another embodiment of the invention, the polynucleotides encoding a splice variant of the invention , or any fragment or complement thereof, may be used for therapeutic purposes. As well, complements of the human voltage-gated calcium channel α2δ-2 subunit isoform encoding nucleic acid molecules can be useful as antisense oligonucleotides, e.g., by delivering the antisense oligonucleotide to an animal to induce a human voltage-gated calcium channel α2δ2-a subunit "knockout" phenotype. The administration of antisense RNA probes to block gene expression is discussed in Lichtenstein, C., Nature 333:801-802 (1988).

Thus, an aspect of the invention features using the complement of the polynucleotide encoding a splice variant of the invention in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding a splice variant of the invention. Thus, complementary molecules or fragments may be used to modulate α2δ-2 subunit protein isoforms, e.g., α2δ2-a of SEQ ID NO:2 activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding α2δ-2 subunit isoform of the invention, e.g., α2δ2-a.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors which will express nucleic acid sequences complementary to the polynucleotides of the gene encoding any one or both of the α2δ-2 subunit isoform detailed herein. See, e.g., Sambrook, supra: and Ausubel, supra.

For example, genes encoding a α2δ2-a subunit detailed herein can be turned off by transforming a cell or tissue with expression vectors which express high levels of a

polynucleotide, or fragment thereof. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding $\alpha 2\delta$-2 subunit isoform detailed herein , e.g., SEQ ID NO:1. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J. E. et al. (1994) in Huber, B. E. and B. I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, N.Y., pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes. As well, using the nucleotide sequences disclosed herein, (SEQ ID NO:1 or 3) one may also design oligoes useful for the silencing of a gene containing a sequence of nucleotides substantially as depicted in one of SEQ ID NO.1 or 3 using RNAi technology. See, WO 01/70949 which teaches methods for silencing genes, which is incorporated by reference herein in its entirety including the references disclosed therein.

Any of the therapeutic methods described herein may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Another aspect of the invention is directed to the use of synthetic oligonucleotides, or other antisense chemical structures designed to bind to mRNA encoding either one of the herein disclosed $\alpha 2\delta$-2 isoforms or a equivalent nucleic acid molecule having a sequence of nucleotides substantially the same as detailed herein (SEQ ID NO:1 or 3) and inhibit translation of mRNA thereby inhibiting expression of encoded gene product. Significantly, such antisense molecules will allow the skilled artisan the means to alter levels of expression of either on of the splice variant polypeptides or a structurally related polypeptide in a suitable host cell.

To be effective, the SAO may be designed so as to be able to pass through cell membranes in order to enter the cytoplasm of the cell. This is made possible by virtue of physical and chemical properties of the SAO which render it capable of passing through cell membranes (e.g. by designing small, hydrophobic SAO chemical structures) or by virtue of

specific transport systems in the cell which recognize and transport the SAO into the cell. Likewise, the SAO can be designed so that upon administration it is directed to selected cell populations. For example, the SAO may be designed to bind to the α2δ2-a subunit sequence which are found only in certain cell types. Alternatively, the SAO may be designed so that it only recognizes and thus selectively binds to the target mRNA sequence, comprising a nucleotide sequence corresponding to a nucleotide sequence contained, for example, in the sequence set forth in SEQ ID NO:1. This object is achieved by virtue of complementary base pairing to the mRNA. The SAO may also be designed to inactivate the target mRNA sequence by any one or more of three mechanisms – (a) binding to the target mRNA and thus inducing degradation of the mRNA by intrinsic cellular mechanisms such as RNAse H digestion; (b) inhibiting translation of the target mRNA by interfering with the binding of translation-regulating factors or of ribosomes; or (c) inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups sufficient to either degrade or chemically modify the target mRNA. Significantly, synthetic antisense oligonucleotide drugs capable of successfully achieving the above referenced objects via modulating mRNA targets are detailed in the prior art. See, e.g., Cohen (1989) Trends in Pharm. Sci. 10:435; and Weintraub (1990) Sci. Am. 262:40-46. Also see, Sarver et al. (1990) Science 247:1222, have reported the successful coupling of ribozymes to antisense oligonucleotides as a promising strategy for inactivating target mRNA.

A vector expressing the complement of the polynucleotide encoding the α2δ2-a subunit polypeptide isoform may be administered to a subject to treat or prevent any one of the disorders described elsewhere in this application.

In accordance with the above, an aspect of the invention is drawn to a human voltage-gated calcium channel α2δ-2 isoform inhibitor, e.g., α2δ2-a wherein the inhibitor is an antisense oligonucleotide that selectively binds to the α2δ-2 isoform encoding nucleic acid molecule, e.g., the nucleic acid molecule of the invention or one having a nucleotide sequence that is substantially the same as those detailed herein, in an amount effective to reduce the expression of the encoded gene product. This is desirable in virtually any medical condition wherein a reduction of a human voltage-gated calcium channel α2δ-2 subunit activity is desirable, e.g., voltage-gated calcium influx.

Alternatively, the isolation of the novel genes encoding the splice variant proteins of the invention may be used to detect and quantitate gene expression in biological samples e.g., biopsied tissues in which expression of an α2δ-2 isoform , e.g., α2δ2-a subunit encoding polynucleotides may be correlated with disease. Specifically, the isolation of the α2δ-2 isoform(s) disclosed herein, makes it possible for the artisan to diagnose a disorder characterized

by loss of expression or excessive expression of the either one of the α2δ-2 isoforms disclosed herein. The polynucleotides for use in the diagnostic assays are preferably oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The diagnostic assay may be used to distinguishing between absence, presence, and excess expression of to determine absence, presence, and excess expression of a α2δ-2 isoform of the invention and to monitor regulation of the subject α2δ-2 isoform levels during therapeutic intervention. In addition, the polynucleotides of the invention may also be used to determine the efficacy of a treatment protocol in a patient undergoing therapy for a one of the many human voltage-gated calcium channel mediated pathologies that are responsive to treatment with the isoform encoding polynucleotides of the invention.

These methods involve determining expression of the nucleic acid molecule encoding said subunit polypeptide, e.g., SEQ ID NO:1, and/ or the gene product thereof (SEQ ID NO:2). In the former situation, such determinations can be carried out via any standard nucleic acid determination assay, including the polymerase chain reaction, or assaying with labeled hybridization probes.

A representative diagnostic method detailing the use of the polynucleotide(s) for diagnostic purposes proposes utilizing PCR probes derived from the sequences detailed herein, for hybridization complexes as a means to identify nucleic acid sequences which encode a protein of SEQ ID NO:2 or a biologically equivalent protein. The probes are capable of detecting polynucleotide sequences having a sequence that is substantially the same as those detailed herein, including genomic sequences. Further, the probes of the invention may also be used for detecting related sequences, and should preferably contain at least 50% of the nucleotides from any of the α2δ2-a encoding sequences. In the method, the specificity of the probe, and/or the stringency of the hybridization or amplification (maximal, high, intermediate, or low) will be determinative of whether the probe identifies only naturally occurring sequences encoding α2δ2-a subunit polypeptide, alleles, or related sequences. The hybridization probes of the subject invention may be DNA or RNA and derived from the nucleotide sequence of SEQ ID NO:1 or 3 from genomic sequence including promoter, enhancer elements, and introns of the naturally occurring α2δ2-a subunit.

In order to provide a basis for the diagnosis of disease associated with aberrant expression of a human α2δ-2 isoform of the invention, a "normal" or standard profile for expression is established. Normal levels may be obtained by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a polynucleotide sequence of the invention or a fragment thereof, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal

subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from subjects who are symptomatic for disease. Deviation between standard and subject values is used to establish the presence of disease.

Alternatively, standard values for a $\alpha 2\delta 2$-a subunit polypeptide expression, e.g., SEQ ID NO:2 , can be established by, combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with an antibody to, for example, $\alpha 2\delta 2$-a subunit of the invention, under conditions favoring the formation of complex therebetween. The amount of standard complex formation may be quantified by various methods, but preferably by photometric, means. Quantities of the $\alpha 2\delta 2$–a subunit polypeptide expressed in control and disease samples from biopsied tissues or biological samples are compared with the standard values. Deviation between standard and subject values, in turn, will establish the parameters for diagnosing the disease.

A variety of protocols for detecting and measuring the expression of $\alpha 2\delta 2$-a subunit polypeptide, using either polyclonal or monoclonal antibodies specific for the protein, are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on $\alpha 2\delta 2$-a subunit polypeptide is preferred, but a competitive binding assay may be employed. These and other assays are well described in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul, Minn., Section IV; and Maddox, D. E. et al. (1983) J. Exp. Med. 158:1211-1216).

Methods for quantifying the expression of the target, e.g., polynucleotides or protein include radiolabeling or biotinylating nucleotides, co-amplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated. (See, e.g., Melby, P. C. et al. (1993) J. Immunol. Methods, 159:235-244; and Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) Formats are available that speed up the quantitation of multiple samples, e.g., running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or calorimetric response provides rapid quantitation.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

The present invention also provides various methods for determining whether a compound/agent can modulate the activity of at least one of the herein disclosed $\alpha 2\delta$-2 subunit polypeptide isoforms, e.g., $\alpha 2\delta 2$-a. The compound can be a substantially pure compound of synthetic origin combined in an aqueous medium, or the compound can be a naturally occurring material such that the assay medium is an extract of biological origin, such as, for example, a plant, animal, or microbial cell extract. A general method proposes contacting $\alpha 2\delta 2$-a subunit or fragments thereof, with the compound under suitable conditions and subsequently determining if the compound modulates the activity of the $\alpha 2\delta 2$-a subunit. The compounds of interest can function as agonists or antagonists of $\alpha 2\delta 2$-a subunit activity. $\alpha 2\delta 2$-a subunit or fragments thereof, can be expressed on a cell or tissue, naturally or recombinantly, or immobilized by attachment to a solid substrate, e.g., nitrocellulose or nylon membrane, glass, beads, etc. Modulators of the $\alpha 2\delta 2$-a subunit isoform of the invention may selectively inhibit or increase $\alpha 2\delta 2$-a subunit polypeptide function based on the state of depolarization of the membrane with which the $\alpha 2\delta 2$-a subunit is associated.

Also provided are efficient methods for identifying pharmacological agents or lead compounds for agents useful in the treatment of conditions associated with aberrant voltage-gated calcium influx mediated by a human voltage-gated calcium channel including the splice variants of the invention. Compounds and agents so identified are also within the scope of the invention.

A variety of assays for pharmacological agents are known, e.g., labeled *in vitro* protein binding assays, Ca^{2+} influx assays, etc. For example, calcium influx assays can be performed to screen and/or determine whether the $\alpha 2\delta 2$-a subunit inhibitor has the ability to inhibit $\alpha 2\delta 2$-a subunit activity, and whether the inhibition is selective. As an example, protein binding screens are routinely used to rapidly examine the binding of candidate pharmacological agents to a target protein, e.g., the $\alpha 2\delta 2$-a subunit of the invention. The candidate pharmacological agents can be derived from, for example, combinatorial peptide libraries. Convenient reagents for such assays are known in the art.

The $\alpha 2\delta$-2 isoform polypeptides, for use in the methods of the invention can be added to an assay mixture as an isolated polypeptide (where binding of a candidate

pharmaceutical agent is to be measured) or as a cell or other membrane preparation containing a functional human voltage-gated calcium channel subunit polypeptide. In the latter assay configuration, the cells contain the $\alpha 2\delta$-2 isoform, e.g., $\alpha 2\delta 2$-a subunit as a preloaded polypeptide or as a nucleic acid (e.g. a cell transfected with an expression vector comprising the nucleotide sequence as shown in SEQ ID NO:1). The polypeptide can be produced recombinantly, or isolated from biological extracts, but preferably is synthesized *in vitro*. Polypeptides for use include the novel $\alpha 2\delta$-2 isoforms of the invention as well as chimeric proteins comprising a fusion of an $\alpha 2\delta$-2 isoform of the invention e.g., the $\alpha 2\delta 2$-a subunit polypeptide with another polypeptide, e.g., a polypeptide capable of providing or enhancing protein-protein binding, or enhancing stability of the $\alpha 2\delta 2$-a subunit polypeptide under assay conditions. A polypeptide fused to the $\alpha 2\delta 2$-a subunit polypeptide or fragment thereof may also provide means of readily detecting the fusion protein, e.g., by immunological recognition or by fluorescent labeling.

The assay mixture further comprises a candidate pharmacological agent. Typically, a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a different response to the various concentrations. Generally, one of these concentrations serves as a negative control, i.e., at zero concentration of agent or at a concentration of agent below the limits of assay detection. Candidate agents include numerous chemical classes, although typically they are organic compounds. Preferably, the candidate pharmacological agents are small organic compounds, i.e., those having a molecular weight of more than 50 yet less than about 2500. In instances, where the agent is a nucleic acid molecule, the agent typically is a DNA or RNA molecule, although modified nucleic acid molecules having non-natural bonds or subunits are also contemplated. Likewise, the agent may be an antibody composition.

A variety of other reagents also can be included in the mixture. These include reagents such as salts, buffers, neutral proteins (e.g., albumin), detergents, etc. which may be used to facilitate optimal protein-protein and/or protein-nucleic acid binding. Such a reagent may also reduce non-specific or background interactions of the reaction components. Other reagents that improve the efficiency of the assay such as protease inhibitors, nuclease inhibitors, antimicrobial agents, and the like may also be used.

The mixture composed of the foregoing assay materials is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the representative human voltage-gated calcium channel $\alpha 2\delta$-2 subunit isoform of the invention transduces a control amount of voltage-gated calcium influx. For determining the binding of a candidate pharmaceutical agent to the $\alpha 2\delta 2$-a subunit, the mixture is incubated under conditions

which permit binding. The order of addition of components, incubation temperature, time of incubation, and other parameters of the assay may be readily determined. Of significant importance is the observation that such experimentation merely involves optimization of the assay parameters, not the fundamental composition of the assay. Following incubation, the level of voltage-gated calcium influx or the level of specific binding between the $\alpha 2\delta 2$-a subunit polypeptide and the candidate pharmaceutical agent is detected by any convenient method available to the user.

For cell free binding type assays, a separation step is often used to separate bound from unbound components. The separation step may be accomplished in a variety of ways. Although it is preferable that at least one of the components is immobilized on a solid substrate, from which the unbound components may be easily separated. The solid substrate can be made of a wide variety of materials and in a wide variety of shapes, e.g., microtiter plate, microbead, dipstick, resin particle, etc. The substrate preferably is chosen to maximize signal to noise ratios, primarily to minimize background binding, as well as for ease of separation and cost. Separation may be effected for example, by removing a bead or dipstick from a reservoir, emptying or diluting a reservoir such as a microtiter plate well, rinsing a bead, particle, chromatographic column or filter with a wash solution or solvent. The separation step preferably includes multiple rinses or washes. For example, when the solid substrate is a microtiter plate, the wells may be washed several times with a washing solution, which typically includes those components of the incubation mixture that do not participate in specific bindings such as salts, buffer, detergent, non-specific protein, etc. Where the solid substrate is a magnetic bead, the beads may be washed one or more times with a washing solution and isolated using a magnet. See U.S. Patent Publication No. 2002/0147309 for details regarding factors that may effect separation.

Detection may be realized in any convenient way for cell-based assays such as a calcium influx assay, *supra*. The calcium influx resulting from voltage stimulus of the $\alpha 2\delta 2$-a subunit polypeptide typically alters (directly or indirectly) a detectable product, e.g., a calcium sensitive molecule such as fura-2-AM.

For cell free binding assays, one of the components usually comprises, or is coupled to, a detectable label. A wide variety of labels can be used, such as those that provide direct detection (e.g., radioactivity, luminescence, optical or electron density, etc) or indirect detection (e.g., epitope tag such as the FLAG epitope, enzyme tag such as horseradish peroxidase, etc.). The label may be bound to a human voltage-gated calcium channel $\alpha 2\delta 2$-a subunit polypeptide or the candidate pharmacological agent.

A variety of methods may be used to detect the label, depending on the nature of the label and other assay components. For example, the label may be detected while bound to the solid substrate or subsequent to separation from the solid substrate. Labels may be directly detected through optical or electron density, radioactive emissions, nonradiative energy transfers, etc. or indirectly detected with antibody conjugates, streptavidin-biotin conjugates, etc. Methods for detecting the labels are well known in the art.

An exemplary cell-based assay of calcium influx involves contacting a neuronal cell expressing a $\alpha 2\delta$-2 isoform having a sequence of amino acids substantially as depicted in SEQ ID NO:2 or a biologically active fragment thereof or a structurally related protein, as determined by percent sequence identity of the primary sequence, e.g., 85% sequence identity and exhibiting a common calcium channel activity with a candidate pharmacological agent under conditions whereby the influx of calcium can be stimulated by application of a voltage to the test system, i.e., by membrane depolarization. Specific conditions are well known in the art and are described in Lin et al., Neuron 18:153-166, 1997. Examples of such methods are also described in U.S. Pat. No. 5,429,921. A reduction in the voltage-gated calcium influx in the presence of the candidate pharmacological agent indicates that the candidate pharmacological agent reduces the induction of calcium influx of the $\alpha 2\delta$-2 isoform subunit in response to the voltage stimulus. An increase in the voltage-gated calcium influx in the presence of the candidate pharmacological agent indicates that the candidate pharmacological agent increases the induction of calcium influx of the $\alpha 2\delta$-2 isoform subunit in response to the voltage stimulus. Methods for determining changes in the intracellular calcium concentration are known in the art.

An amount of the $\alpha 2\delta$-2 isoform subunit inhibitor which is effective to inhibit voltage-gated calcium influx in the mammalian cell is an amount which is sufficient to reduce voltage-gated calcium influx by at least 10%, preferably at least 20%, more preferably 30% and still more preferably 40%. For example, an amount of a human voltage-gated calcium channel $\alpha 2\delta 2$–a subunit which is effective to increase voltage-gated calcium influx in the mammalian cell is an amount which is sufficient to increase voltage-gated calcium influx by at least 10%, preferably at least 20%, more preferably 30% and still more preferably 40%. Such alterations in voltage-gated calcium influx can be measured by the assays described herein.

According to the invention $\alpha 2\delta$-2 isoform inhibitors also include "dominant negative" polypeptides derived for example from SEQ ID NO:2 or 4. A dominant negative polypeptide refers to an inactive variant of a polypeptide, which, by interacting with the cellular machinery, displaces an active polypeptide from its interaction with the cellular machinery or competes with the active polypeptide, thereby reducing the effect of the active polypeptide. For example, a dominant negative receptor which binds a ligand but does not transmit a signal in

response to binding of the ligand can reduce the biological effect of expression of the ligand. Likewise, a dominant negative human voltage-gated calcium channel α2δ-2 isoform of an active complex (e.g. voltage-gated calcium channel) can interact with the complex but prevent the activity of the complex (e.g. voltage-gated calcium influx).

The end result of the expression of a dominant negative polypeptide, e.g., a α2δ2-a subunit polypeptide of the invention in a cell is a reduction in voltage-gated calcium influx. One of ordinary skill in the art can assess the potential for a dominant negative α2δ2-a subunit polypeptide, and using standard mutagenesis techniques to create one or more dominant negative "altered" polypeptides. For example, one of ordinary skill in the art can modify the sequence of the human voltage-gated calcium channel α2δ2-a subunit polypeptide (SEQ ID NO:2) by site-specific mutagenesis, scanning mutagenesis, partial gene deletion or truncation, and the like. See, Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989. The skilled artisan then can test the population of mutagenized polypeptides for diminution in α2δ2-a subunit activity (e.g., voltage-gated calcium influx) and/or for retention of such an activity. Other similar methods for creating and testing dominant negative altered polypeptides of an isoform of the invention will be apparent to one of ordinary skill in the art.

Methods for reducing or inhibiting α2δ-2 isoform activity, e.g. α2δ2-a subunit activity in a cell are also encompassed by the present invention. The method proposes contacting the mammalian cell with an amount of a α2δ2-a subunit inhibitor effective to inhibit voltage-gated calcium influx in the mammalian cell. Such methods are useful *in vitro* for altering voltage-gated calcium influx for the purpose of, for example, elucidating the mechanisms involved in stroke, pain, e.g., neuropathic pain, and traumatic brain injury and for restoring the voltage-gated calcium influx in a cell having a defective α2δ2-a subunit. *In vivo*, such methods are useful, for example, for reducing voltage-gated voltage-gated calcium influx, e.g., to treat stroke, pain, e.g., neuropathic pain, traumatic brain injury, or any condition in which the herein disclosed human voltage-gated calcium channel α2δ2-1 subunit activity is elevated.

The invention also encompasses a method for increasing expression of a human voltage-gated calcium channel α2δ-2 isoform e.g., α2δ2-a subunit in a cell or subject, especially is those subjects having a disorder characterized by a deficiency in voltage-gated calcium influx. The method proposes contacting the cell with, or administering to the subject, an effective amount of α2δ2-a subunit encoding nucleic acid or the polypeptide encoded thereby sufficient to increase voltage-gated calcium influx in the cell or the subject. An increase in α2δ2-a subunit activity, for example, can be measured by the assays described herein, e.g., assays of calcium influx. These assays are conducted according to standard procedures of testing calcium channel

function (e.g. patch clamping, fluorescent Ca^{2+} influx assays) which require no more than routine experimentation. Using such methods, modulators of the activity of the $\alpha 2\delta$-2 isoforms of the invention effective at a particular voltages (e.g. complete membrane depolarization) can also be identified. Such compounds, in turn, will find use in selectively modulating calcium channel activity in conditions which may display voltage dependence. For example, following a stroke membranes are generally depolarized and such compounds may be active in selectively blocking calcium channel activity for treatment of stroke. Other uses will be apparent to one of ordinary skill in the art.

In another aspect, the invention provides for a method of tracking a neurodegenerative disorder characterized by aberrant calcium influx, e.g., epilepsy, comprising administering to a patient in need thereof. Thereof, a therapeutically effective amount of an antiepileptic compound/formulation sufficient to alleviate said disorder or alternatively sufficient to correct said aberrant calcium influx. The above is premised in part on the finding by the inventors that the herein disclosed $\alpha 2\delta 2$ isoforms, $\alpha 2\delta 2$-a and $\alpha 2\delta 2$-b bind gabapentin with high affinity in conformance with the findings of Gee et al., supra. Consequently, the antiepileptic drug detailed in Gee, et al., supra, or a class of compounds having a similar pharmacology and binding properties will also find use in the treatment of voltage activated calcium channel mediated disorders.

The invention also contemplates *ex vivo* gene therapy, a preferred procedure for which is detailed in U.S. Pat. No. 5,399,346 and in references cited therein. A representative example of such therapy proposes introducing *in vitro* a functional copy of a gene, e.g., SEQ ID NO:1 or 3 , into a cell(s) of a subject in need of such therapy, e.g., subject containing a defective copy of the gene, and returning the genetically engineered cell(s) to the subject. It is noted that the functional copy of the gene is under operable control of regulatory elements that favor expression of the gene in the genetically engineered cell(s). *In vivo* gene therapy is also encompassed using appropriate vectors such as adenovirus, retroviruses, herpes virus, and targeted liposomes.

It is recognized that the preparations of the invention should be administered in effective amounts. An effective amount defines that amount of a pharmaceutical preparation that alone, or together with further doses, produces the desired response. In the case of treating a condition characterized by aberrant voltage-gated calcium influx, the desired response is reducing or increasing calcium influx to a level which is within a normal range. Preferably, the change in calcium influx produces a detectable reduction in a physiological function related to the condition, e.g., a reduction in neurotoxicity following an epileptic attack or stroke. The responses can be monitored by routine methods. In the case of a condition where an increase in

voltage-gated calcium influx is desired, an effective amount defines that amount necessary to increase said influx in the target tissue. The converse is the case when a reduction in influx is desired. An increase or decrease in neurotransmitter release also could be measured to monitor the response.

The amount to be delivered will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. It is preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. It is also recognized that lower doses will result from other forms of administration, such as intravenous administration. As well, multiple doses are also contemplated to achieve appropriate systemic levels of compound, although fewer doses typically will be given when compounds are prepared as slow release or sustained release medications.

When administered, the pharmaceutical preparations of the invention are preferable applied in pharmaceutically-acceptable amounts and in pharmaceutically-acceptably compositions. Such preparations may routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

Inhibitors of the $\alpha 2\delta$-2 isoforms detailed herein, may be combined, optionally, with a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is

combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy. The pharmaceutical compositions may contain suitable buffering agents, including: acetic acid in a salt; citric acid in a salt; and phosphoric acid in a salt. As well, the pharmaceutical compositions may contain, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.

The invention contemplates a variety of administration routes. The particular mode selected will depend, of course, upon the particular compound selected, the severity of the condition being treated and the dosage required for therapeutic efficacy. Suffice it to say that the methods of the invention may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of the active compounds without causing clinically unacceptable adverse effects. Such modes of administration include oral, rectal, topical, nasal, interdermal, or parenteral routes. The term "parenteral" includes subcutaneous, intravenous, intrathecal, intramuscular, or infusion. Intravenous or intramuscular routes are not particularly suitable for long-term therapy and prophylaxis. In addition, the pharmaceutical compositions of the invention may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the active compound. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

Compositions suitable for parenteral administration generally comprise a sterile aqueous preparation of one of the human voltage-gated calcium channel $\alpha 2\delta 2$-a subunit inhibitor or the human voltage-gated calcium channel $\alpha 2\delta 2$-a subunit nucleic acid molecules and polypeptides, which is preferably isotonic with the blood of the recipient. This aqueous preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils

are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Carrier formulation suitable for oral, subcutaneous, intravenous, intrathecal, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa.

Other delivery systems can include time-release, delayed release or sustained release delivery systems such as the biological/chemical vectors is discussed above. Such systems can avoid repeated administrations of the active compound, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. Use of a long-term sustained release implant may be desirable. Long-term release, are used herein, means that the implant is constructed and arranged to delivery therapeutic levels of the active ingredient for at least 30 days, and preferably 60 days. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above.

Another aspect of the invention features use of the novel polynucleotides, encoded $\alpha_2\delta$-2 encoded isoforms in a variety of diagnostic method kits. Typically the kit will have a compartment containing either a defined $\alpha_2\delta$-2 subunit isoform of the invention, e.g., a $\alpha_2\delta$2-a polypeptide, polynucleotide, or a reagent which recognizes one or the other, e.g., antigen fragments or antibodies. Additionally the kit will include the reagents needed to carry out the assay in a separate compartment as well as instructions for use and proper disposal.

Use of an agent identified as described herein in an appropriate animal model is also within the scope of this invention. Thus, for example, an agent identified as described herein (e.g., the polynucleotides and encoded proteins of the invention, a $\alpha_2\delta$2-a or -b modulating agent, an antisense $\alpha_2\delta$2-a or -b nucleic acid molecule, a $\alpha_2\delta$2-a or -b-specific antibody, or a $\alpha_2\delta$2-a or –b binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent.

In accordance with the above, an aspect of the invention embraces using the novel polynucleotides of the invention to prepare a non-human transgenic animal. A "transgenic animal" is an animal having cells that contain DNA which has been artificially inserted into a cell, which DNA becomes part of the genome of the animal that develops from that cell. Preferred transgenic animals are primates, mice, rats, cows, pigs, horses, goats, sheep, dogs and cats. Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Charles River (Wilmington, Mass.), Taconic (Germantown, N.Y.), Harlan Sprague Dawley (Indianapolis, Ind.), etc. Transgenic animals having a particular property associated with a particular disease can be used to study the affects of a variety of drugs and

treatment methods on the disease, and thus serve as effective genetic models for the study of a number of human diseases. The $\alpha_2\delta$2-a subunit knockout and transgenic animals, for example, may be used as models for the study of disorders involving voltage-gated calcium influx or dysfunctional calcium channels. A variety of methods are available for the production of transgenic animals associated with this invention. See e.g., Brinster et al., Proc. Nat. Acad. Sci. USA, 82: 4438 (1985); Brinster et al., Cell 27: 223 (1981); Costantini et al., Nature 294: 982 (1981); Harpers et al., Nature 293: 540 (1981); Wagner et al., Proc. Nat. Acad. Sci. USA 78:5016 (1981); Gordon et al., Proc. Nat. Acad. Sci. USA 73: 1260 (1976).

EXAMPLES

Some of the standard methods are described or referenced, e.g., in Maniatis et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols. 1-3, CSH Press, N.Y.; or Ausubel et al. (1987 and Supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York; Innis et al. (eds.) (1990) PCR Protocols: A Guide to Methods and Applications Academic Press, N.Y. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification" in Methods in Enzymology, vol. 182, and other volumes in this series; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, Calif. Combination with recombinant techniques allow fusion to appropriate segments, e.g., to a FLAG sequence or an equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering Principle and Methods 12:87-98, Plenum Press, N.Y.; and Crowe et al. (1992) OIAexpress: The High Level Expression & Protein Purification System QIAGEN, Inc., Chatsworth, Calif.

EXAMPLE 1

Isolation/Characterization of the novel variants of the invention

$\alpha_2\delta$-2 sequences were amplified by standard PCR techniques.

Splice variant #1 (+Q) was originally identified in a human thyroid carcinoma cell line (TT cells). First strand cDNA was synthesized from TT cell total RNA with the

RETROscript kit (Ambion) according to the manufacturer's instructions. A sense strand 25-mer, CAGTGGATGGCCTGTGCCAACAAAG (corresponds to nts 1315-1339 of the reference sequence coding region) and an antisense 22-mer, GGTGCTCAGAGGCGGCGAGAGG (corresponds to nts 3422-3443 of the reference sequence coding region), were used in amplification reactions with TT cDNA and *Pfu*Turbo DNA polymerase (Stratagene). Reactions were performed at 94°C for 5 min, followed by 35 cycles of 94°C for 20 sec, 58°C for 20 sec and 72°C for 2.5 min, and one additional cycle at 72°C for 7 min.

Splice variant #2 was amplified from DNA isolated from a human DRG cDNA library with a sense strand 22-mer, CAGTGGATGGCCTGTGCCAACAAAG (corresponds to nts 2607-2628 of the reference sequence coding region), an antisense 22-mer, GGTGCTCAGAGGCGGCGAGAGG (corresponds to nts 3422-3443 of the reference sequence coding region) and *Pfu*Turbo DNA polymerase (Stratagene). Reactions were performed at 95°C for 5 min, followed by 35 cycles of 95°C for 20 sec, 60°C for 20 sec and 72°C for 1.75 min, and one additional cycle at 72°C for 7 min. The sizes of the amplified products, an ~2130 bp fragment containing splice variant #1 and an ~900 bp fragment containing splice variant #2, were initially determined by gel electrophoresis. The amplification products were subcloned into the bacterial plasmid pCRBluntII with the Zero Blunt TOPO PCR Cloning kit (Invitrogen) according to the manufacturer's instructions and the nucleotide sequence of multiple isolates determined with an Applied Biosystems 3100 automated sequencer.

EXAMPLE 2

α2δ2-a and –b splice variant transfection, membrane preparation and binding assay

Materials and methods

For transient transfections, HEK-293 or COS-7 host cell lines (ATCC) were plated at a density of 5-6 million cells/10 cm poly-D-lysine pre-coated plate (Becton Dickinson). Transfections were performed with 10-20 μg of α2δ2-a or α2δ2-b cDNA's in pCDNA3 vector (Invitrogen, Carlsbad) using the Lipofectamine 2000 reagent, according to manufacturer's directions: (http://www.invitrogen.com/content/sfs/manuals/lipofectamine2000_man.pdf).

At 48 hours, cells were harvested by scraping into isotonic PBS (Invitrogen Gibco), pelleting at 400 *g* for 5 minutes, decanting the supernatant and adding 25 ml of 20 mM HEPES, 10 mM EDTA; pH 7.4 and homogenizing the cells with a Polytron tissue homogenizer (Kinematica, Switzerland) at medium setting for 15-20 seconds. Membranes were pelleted at

40, 000 g for 10 minutes at 4°C (Sorvall SS-34 rotor 18,500 r.p.m.) and the process was repeated. The buffer was changed to 25 ml of 20 mM HEPES, 0.1 mM EDTA; pH 7.4 and the process was repeated twice more. The resulting pellet was suspended in 20 mM HEPES, 0.1 mM EDTA and homogenized to give an approximate concentration of about 1 mg/ml protein, as determined by the Pierce BCA protein kit, according to the manufacturer's directions: (http://www.piercenet.com/files/1296dh4.pdf). Membranes were stored at -70°C until used.

When used, membranes were thawed on ice and re-homogenized with a Polytron in 20 mM HEPES; pH 7.4, once, and assay buffer (20 mM HEPES; pH 7.4 + 2 mM (final concentration) $MgCl_2$) at least twice, and resuspended at a final concentration of 0.2 mg/ml (100 µg / 500 µl reaction volume). ^{3}H-gabapentin (Merck-Rahway, or Amersham, Piscataway, NJ) was added to a final concentration of 7 nM, and various amounts (10 pM to 10 uM) of unlabeled gabapentin displacer (Merck Frosst) were added to create the dose-response curve, which was performed in triplicate for each assay. Binding was allowed to proceed to equilibrium (at least 90 minutes) at room temperature and membranes were harvested using a 96-well harvester (Brandel, Gaithersburg, MD) onto GF/B filter plates (Perkin-Elmer, Boston, MA) that had been presoaked in 0.5% (v/v) polyethylenimine (Sigma, St. Louis) for at least 30 minutes. Filter plates were washed several times with 100-200 ml of assay buffer and the plates were dried. Approximately 50 µl of scintillation fluid (Microscint 20, Packard Biosciences, Meriden, CT) was added to each well and incubated for at least an at room temperature. Plates were read for 2 minutes per well in a TopCount (Packard) reader.

Data obtained from the TopCount was imported into Excel (Microsoft) and any triplicate points that were more than 20% above or below the average of the other two points of a given triplicate were excluded from further analysis. Linefitting and IC_{50} estimation were performed and the goodness of fit values were determined by the Prism software program (GraphPad software), using a variable slope sigmoid curve line, plotted as signal versus the log of the displacer concentration. Assays were run with 'reference $\alpha_2\delta_2$ ' membranes and partially purified $\alpha_2\delta_1$ construct as positive controls. The data is depicted in Figure 1, which clearly shows the specific binding affinity of each of the herein disclosed splice variants for gabapentin compared to the reference.

All patents, patent applications, and publications mentioned herein, whether supra or infra, are each incorporated by reference in its entirety. The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the invention to the specific embodiments described below.